

Table 3. ToxCast *in vitro* HTS Assay Data: 25th Percentile AC50 Values.**(A) 5HPP-33**

Assay Endpoint^a	Description	Endpoint Classification	AC50 (μM)
ATG_ERa_TRANS_up	Increase in ER alpha transactivation in HepG2 cells	Estrogen receptor alpha	0.168
TOX21_ERa_LUC_BG1_Agonist	Agonism against ER 1 in BG1 cells	Estrogen receptor alpha	0.196
ATG_PXR_TRANS_up	Increase in PXR transactivation in HepG2 cells	Xenosensor	0.263
OT_ERa_EREFP_0480	Increase in ERa fluorescent protein induction in HeLa cells	Estrogen receptor alpha	0.306
OT_ERa_EREFP_0120	Increase in ERa fluorescent protein induction in HeLa cells	Estrogen receptor alpha	0.317
ACEA_T47D_80hr_Positive	Effect on cell growth kinetics in T47D cells	Cell growth regulation	0.448
TOX21_ERa_BLA_Agonist_ratio	Induction of ERa beta lactamase in HEK293T cells	Estrogen receptor alpha	0.520
NVS_ENZ_hPI3Ka	Inhibition of human PI3 K enzyme activity (cell-free)	Cell growth regulation	0.572
NVS_NR_mERa	Cell-free binding to the mouse estrogen receptor alpha	Mouse estrogen receptor liganding	0.980
Tox21_p53_BLA_ratio ^b	Activation of p53 signaling pathway in HCT116 cells	Cell cycle checkpoint	1.036
NVS_NR_bER	Cell-free binding to the bovine estrogen receptor alpha	Bovine estrogen receptor liganding	1.062
NVS_NR_hER	Cell-free binding to the human estrogen receptor alpha	Human estrogen receptor liganding	1.337

OT_ER_ERbERb_0480	Increase in ERb fluorescent protein induction in HeLa cells	Estrogen receptor	1.396
OT_ER_ERaERb_0480	Increase in ERa fluorescent protein induction in HEK293T cells	Estrogen receptor	1.618
TOX21_MMP_ratio_down	Decreased membrane potential in HEPG2 cells	Mitochondrial depolarization	2.518
NVS_ENZ_oCOX1	Cell-free enzymatic activity to sheep prostaglandin-endoperoxide synthase 1	Cyclooxygenase-1 activity	2.770
BSK_3C_Proliferation_down	Decrease in proliferation in umbilical vein endothelial cells	Cell growth regulation	2.900
APR_HepG2_p53Act_72h_up	Activation of p53 in HepG2 cells	Cell cycle checkpoint	3.074
BSK_3C_Vis_down	Decrease in visual activity in umbilical vein endothelial cells	Cellular health	3.202
ATG_ERE_CIS_up	Increase in ERS1 activation in HepG2 cells	ER steroid hormone activity	3.625
APR_HepG2_MitoticArrest_24h_up	Increase in mitochondrial arrest at 24 h in HepG2 cells	Cell growth regulation	3.760
BSK_4H_MCP1_up	CCL2 upregulation in vascular umbilical vein endothelial cells	Inflammatory cytokine signals	3.765
ATG_PXRE_CIS_up	Increase in PXR activation in HepG2 cells	Xenosensor	4.064
OT_ER_ERaERa_0480	Agonism against ER 1 in HEK293T cells	ER steroid hormone activity	4.196
NVS_NR_hPXR	Inhibition of human PXR binding (cell-free)	Xenosensor	4.256

CEETOX_H295R_TESTO_dn	Decreased catalytic activity of testosterone in H295R cells	Steroidogenesis	4.377
ATG_DR5_CIS_up	Increase in RAR cis-activation in HepG2 cells	Retinoid signaling	4.386
NVS_ADME_hCYP2C9	Cell-free enzyme activity at human Cyp2C9	Detoxification/metabolism	4.403
NHEERL_ZF_144hpf_TERATOSCORE_up	Increase in TERATOSCORE in zebrafish at 144hpf	Abnormal embryo development	4.440
BSK_3C_IL8_up	Increase in IL-8 protein levels in umbilical vein endothelial cells	Inflammatory cytokine signals	4.528
BSK_LPS_IL8_up	CXCL8 downregulation in vascular umbilical vein endothelial cell/peripheral blood mononuclear cell cocultures	Inflammatory cytokine signals	4.687
APR_HepG2_MitoMass_72h_up	Increase in mitochondrial mass in HepG2 cells	Mitochondrial number	4.746
BSK_hDFCGF_TIMP1_up	Increase in TIMP metalloproteinase inhibitor protein levels in foreskin fibroblast cells	Extracellular matrix remodeling	4.754
NVS_ENZ_oCOX2	Inhibition of sheep COX2 enzymatic activity (cell-free)	cyclooxygenase-2 activity	4.911
TOX21_TR_LUC_GH3_Antagonist	Antagonism of thyroid hormone receptor alpha and beta in GH3 cells	Nuclear receptor activity	5.177
NVS_ADME_hCYP2C19	Inhibition of human CYP2C19 activity (cell-free)	Detoxification/metabolism	5.677

APR_HepG2_CellCycleArrest_24h_dn	Decrease in cell cycle arrest at 24 h in HepG2 cells	Cell growth regulation	5.946
APR_HepG2_CellCycleArrest_72h_dn	Decrease in cell cycle arrest at 72 h in HepG2 cells	Cell growth regulation	6.384
BSK_4H_Pselectin_down	Decrease in P-selectin protein levels in umbilical vein endothelial cells	Endothelial cell-related signaling	6.740
APR_HepG2_StressKinase_24h_up	Increase in stress kinase enzyme activity at 24 h in HepG2 cells	Cellular stress	7.050
BSK_3C_HLADR_down	Decrease in major histocompatibility complex, class II, DR alpha levels in umbilical vein endothelial cells	Immune function	7.101
APR_HepG2_MitoticArrest_72h_up	Increase in cell cycle arrest in HepG2 cells	Cell growth regulation	7.368
BSK_LPS_CD40_down	Decrease in CD40 (TNF receptor superfamily member 5) in vascular umbilical vein endothelial cell/peripheral blood mononuclear cell cocultures	Immune and inflammatory regulation	7.566

(B) TNP-470

Assay Endpoint ^a	Description	Endpoint Classification	AC50 (μM)
BSK_3C_Proliferation_down	Decrease in proliferation in umbilical vein endothelial cells	Cell growth regulation	0.001
Tanguay_ZF_120hpf_YSE_up	Increase in yolk sac developmental defects	Abnormal embryo development	0.003

Tanguay_ZF_120hpf_PE_up	Increase in pericardial edema	Abnormal embryo development	0.009
Tanguay_ZF_120hpf_CFIN_up	Increase in caudal fin developmental defects	Abnormal embryo development	0.009
Tanguay_ZF_120hpf_AXIS_up	Increase in axial developmental defects	Abnormal embryo development	0.018
NVS_GPCR_rGHB	Increase in binding to rat tetraspanin 17 protein, important in cell adhesion, motility, activation and proliferation	G-protein coupled receptor	0.025
Tanguay_ZF_120hpf_EYE_up	Increase in eye developmental defects	Abnormal embryo development	0.034
Tanguay_ZF_120hpf_JAW_up	Increase in jaw developmental defects	Abnormal embryo development	0.040
ATG_PXRE_CIS_up	Increase in PXR activation in HepG2 cells	Xenosensor	0.323
ATG_NF_kB_CIS_dn	Decrease in NFkB activation in HepG2 cells	Immune and inflammatory regulation	0.364
Tanguay_ZF_120hpf_SNOU_up	Increase in snout developmental defects	Abnormal embryo development	0.452
ATG_IR1_CIS_dn	Decrease in 1H4 Farnesoid FXR activation in HepG2 cells	Detoxification/metabolism	1.469
NVS_ADME_hCYP2C19	Cell-free enzyme activity at human Cyp2C19	Detoxification/metabolism	2.032
BSK_4H_Pselectin_down	Decrease in P-selectin protein levels in umbilical vein endothelial cells	Endothelial cell-related signaling	2.048

Tanguay_ZF_120hpf_PFIN_up	Increase in pectoral fin developmental defects	Abnormal embryo development	2.189
NVS_ADME_hCYP3A4	Cell-free enzyme activity at human Cyp3A3	Detoxification/metabolism	2.225
TOX21_ARE_BLA_agonist_ratio	Induction of beta lactamase reporter in HepG2 cells measuring transcriptional activation of ARE	Antioxidant response element	2.459
BSK_hDFCGF_CollagenIII_down	Decrease in Collagen III protein levels in foreskin fibroblast cells	Extracellular matrix remodeling	2.683
BSK_LPS_MCSF_down	Downregulation of macrophage colony stimulating factor 1 in umbilical vein endothelium and peripheral blood mononuclear cells	Inflammatory cytokine signals	3.048
BSK_SAg_IL8_down	Downregulation of chemokine ligand 8 in umbilical vein endothelium and peripheral blood mononuclear cells	Inflammatory chemokine signals	3.153
BSK_SAg_Proliferation_down	Downregulation of proliferation in umbilical vein endothelium and peripheral blood mononuclear cells	Cell growth regulation	3.434

a

Additional assay information downloadable at https://www3.epa.gov/research/COMPTOX/toxcast_assay.html.

b

Assay run multiple times (5) with consistent results (AC50 range 1.04-3.18 µM).