Protocol of AhR HepG2 Cell-based Assay for High-throughput Screening

DOCUMENT: AhR_TOX21_SLP_Version1.0

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ASSAY RFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
Human AhR (Recombinant)	HepG2	Human	Hepatocellular carcinoma	Luciferase reporter	Denison Lab	NR signaling

QUALITY CONTROL PRECAUTIONS:

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
-Alpha-MEM	-Invitrogen	-12561
-Premium FBS	-Atlanta Biologicals	-S11150
-G418	-Invitrogen	-10131-027.
-0.25% trypsin/EDTA	-Invitrogen	-25300
-PBS	-Invitrogen	-14190
-DMSO	-AMRESCO	-KD Medical, RGE-3070
-Recovery cell culture freezing medium	-Invitrogen	-12648
-White solid bottom plates	-Greiner	-
-omeprazole	-Sigma Aldrich	-
-Pintool	-Kalypsis	-
-Viewlux	-PerkinElmer	-
-Centrifuge	-Sorvall legend XTR	-Thermo Fisher Science 75004520
-tetraoctylammonium bromide	-sigma	-88000
-One-Glo	-Promega	-E6130
-celltiter fluor	-Promega	-G6082

PROCEDURE:

1. Cell handling:
   1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
-Alpha MEM	-90%	-90%	-90%	-
-Premium FBS	-10%	-10%	-10%	-
-G418	-400mg/mL	-	-	-
-Recovery cell culture freezing medium	-	-	-	-100%

   1.2. Thawing method

      1.2.1 -place 14mL of pre-warmed thaw media into T75 flask
      1.2.2 -remove vial of cells to be thawed from -140 and thaw rapidly by placing in water bath with gentle agitation for 1-2min
      1.2.3 -Wipe vial with 70% ethanol before opening in biological safety cabinet
      1.2.4 -transfer vial contents dropwise into 10mL of thaw medium in 15mL conical tube
      1.2.5 -centrifuge cells at 1000 rpm for 4min
      1.2.6 -Resuspend and transfer contents into T75 flask containing thaw medium and transfer flask into incubator
      1.2.7 -switch to growth media at first passage

   1.3. Propagation method

      1.3.1 -aspirate media, rinse once with dPBS, add 0.25% trypsin/EDTA and swirl to coat flask evenly
      1.3.2 -add equal volume of growth medium to inactivate trypsin after 2-3 minutes incubation
      1.3.3 -centrifuge cells at 1000 RPM for 4min and resuspend in growth medium before adding to new flask
      1.3.4 -cells should be passaged or fed at least twice per week

2. Assay Protocol

   2.1 -Harvest cells from culture in growth medium and resuspend in assay medium
   2.2 -dispense 4000 cells per well into 1536-well tissue culture treated white solid bottom plate using multidrop dispenser
   2.3 -Incubate cells 5hr, then dispense 23nL of compound, positive control, or DMSO control using pintool
   2.4 -positive and control compounds are located in the first four columns according to the plate map, and library compounds located in columns 5-48
   2.5 -incubate plates for 19hr at 37 degrees C
   2.6 -add 1uL of celltiter fluor to each well using bioraptr dispenser
   2.7 -incubate 30min at 37 degrees C
   2.8 -measure fluorescence using ViewLux
   2.9 -Add 5uL of OneGlo using bioraptr dispenser
   2.10 -incubate 30min at room temperature
   2.11 -Read luminescence on ViewLux

3. Assay Performance

AhR (Omeprazole)	Online Validation Agonist (Mean ± SD)	Online Validation Viability (Mean ± SD)
EC50	33.2 ± 26.0 µM (n = 27)	NA
S/B	4.83 ± 0.55	5.03 ± 0.16
CV (%)*	16.64 ± 4.29 (n = 18)	7.76 ± 0.54 (n = 18)
Z'	0.35 ± 0.09	0.69 ± 0.03

* CV values shown represent average of DMSO plates and low concentration plates only.