Protocol of PXR-Luc HepG2 Cell-based Assay for High-throughput Screening

DOCUMENT: PXR-Luc HepG2_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 PXR	HepG2	Human	Hepatocellular carcinoma	Luciferase reporter	Dr. Chen (St. Jude)	NR Signaling

QUALITY CONTROL PRECAUTIONS:

1. -Maintain cells below 85-90% confluence.

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
-EMEM	-ATCC	-ATCC/30-2003
-Phenol red-free DMEM medium	-Invitrogen	- Invitrogen/31053
-Fetal Bovine Serum	- Hyclone	- Hyclone/SH30071.03
-Charcoal/dextran treated FBS	- Invitrogen	- Invitrogen/12676
-Sodium pyruvate	-Invitrogen	-Invitrogen/11360
-L-Glutamine	- Invitrogen	- Invitrogen/25030
-Penicillin & Streptomycin	-Invitrogen	-Invitrogen/15140
- Geneticin (G418)	- Invitrogen	- Invitrogen/10131
-Recovery Cell culture Freezing Medium	-Invitrogen	-Invitrogen/12648
-0.05% Trypsin-EDTA	-Invitrogen	-Invitrogen/25300
-Rifampicin (Agonist control compound)	-Sigma	-Sigma/R7382
-DL-Sulforaphane (Antagonist control compound)	-Sigma	-Sigma/S4441
-Tetraoctyl ammonium bromide (Viability control compound)	-Sigma	-Sigma/294136
-1536-well white solid plates	-Greiner Bio-One	-Greiner Bio-One / 789173-

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-MULTIDROP COMBI	-Thermo Electron Corporation	-Thermo Electron Corporation
-BioRAPTR FRD	-Beckman Coulter	-Beckman Coulter
-ViewLux Plate Reader	-Perkin Elmer	-Perkin Elmer
-CellTiter-Fluor(TM) Assay System	-Promega	-Promega/G6082
-ONE-Glo(TM) Luciferase Assay System	-Promega	-Promega/E6130

PROCEDURE:

1. Cell handling:
1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
-EMEM	-90%		-90%	
-Phenol red-free DMEM medium		-95%		
-Fetal Bovine Serum	-10%		-10%	
-Charcoal/dextran treated FBS		-5%		
-Sodium pyruvate		-1mM		
-L-Glutamine		-2mM		
-Penicillin & Streptomycin	-100U/ml & 100ug/ml	-100U/ml & 100ug/ml	-100U/ml & 100ug/ml	
- Geneticin (G418)	-500ug/ml			
-Recovery Cell culture Freezing Medium				-100%

1.2. Thawing method

1.2.1 -Thaw a vial of cells in 9ml of pre-warmed thaw medium and then centrifuge
1.2.2 –Re-suspend the pellet with the thaw medium and seed at 2 million cells per T-75 flask

1.3. Propagation method

1.3.1 -Trypsinize cells from the culturing flask and centrifuge and then re-suspend cells in culture medium
1.3.2 -Passage cells at 2-3 million per T-225 flask

2. Assay Protocol

2.1 -Trypsinize cells from the culturing flask and centrifuge and then re-suspend cells in assay medium at a density of 0.4 X 10^6 cells/mL
2.2 -Dispense 2000 cells/5uL/well into 1536-well tissue treated white/solid bottom plates using a 8 tip dispenser (Multidrop)
2.3 -Incubate the plates for 5hr at 37C and 5% CO2
2.4 -Transfer 23nL of compounds from the library collection (0.59nM to 92uM) and positive control through Pintool
2.5 -Incubate the plates for 24hr at 37C and 5% CO2
2.6 –After 23hrs of incubation at 37C, add 1ul of CellTiter-Fluor using single tip dispense (Bioraptr)
2.7 -Incubate the plates for 1hr at 37C and 5% CO2
2.8 –Read fluorescence (exposure time = 3sec) intensity using ViewLux plate reader
2.9 -Then add 4ul of ONE-Glo(TM) Luciferase reagent using a single tip dispense (Bioraptr)
2.10-Incubate the plates at room temperature for 30min
2.11- Read fluorescence (exposure time = 45sec) intensity using ViewLux plate reader

3. Assay Performance

Online Validation	PXR-Luc HepG2 Agonist mode	PXR-Luc HepG2 Viability-Agonist
CV*	9.73 ± 0.76 (n = 21)	4.33 ± 0.45 (n = 24)
S/B	4.84 ± 0.54	5.75 ± 0.09
Z	0.66 ± 0.07	0.85 ± 0.02

♂*CV values shown represent average of all plates excluding high compound concentration plates.