# 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-F |
| -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.