Protocol of Auto Fluorescence HepG2 and HEK293 Cell-based Assay for High-throughput Screening

DOCUMENT: Auto Fluorescence_TOX21_SLP_Version1.0

TITLE: Protocol of Auto Fluorescence HepG2 and HEK293 Cell-based Assay for High-throughput Screening

ASSAY RFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
Auto fluorescence of the compounds	HepG2 and HEK293	Human	Hepatocellular carcinoma and Embryonic kidney	Fluorescence Intensity	-	-

QUALITY CONTROL PRECAUTIONS:

1. -The assay should be performed in black-clear bottom 1536 well plates, so the bottom of the plates should not be touched
2. -Cell culture is maintained by passaging twice a week and should not reach more than 90% confluence
3. -Only the top 5 odd concentrations of the first day sets (NTP, EPA and NCTT) of compound plates were used for transferring to the assay plates

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
-Eagle's Minimum Essential Medium	-ATCC	-ATCC / 30-2003
-Fetal Bovine Serum	-Hyclone	-Hyclone / SH30071.03
-Penicillin and Streptomycin	-Invitrogen	-Invitrogen / 15140
-0.25% Trypsin-EDTA	-Invitrogen	-Invitrogen / 25200
-Recovery Cell Culture Freezing Medium	-Invitrogen	-Invitrogen / 12648
-Black-clear bottom 1536 well plates	-Greiner	-Greiner / 789092F
-Multidrop COMBI	-Thermo Electron Corporation	-Thermo Electron Corporation
-Envision Plate Reader	-Perkin Elmer	-Perkin Elmer
-Fluorescein (Green channel control compound)	-Sigma	-Sigma/F2456

-Triamterene (Blue channel control compound)	-Sigma	-Sigma/T4143
-Rose Bengal sodium (Red channel control compound)	-Sigma	-Sigma/11950

PROCEDURE:

1. Cell handling:
   1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
-Eagle's Minimum Essential Medium	-90%	-90%	-90%	-
-Fetal Bovine Serum	-10%	-10%	-10%	-
-Penicillin and Streptomycin	-100U/ml and 100ug/ml	-100U/ml and 100ug/ml	-100U/ml and 100ug/ml	-
-Recovery Cell Culture Freezing Medium	-	-	-	-100%

   1.2. Thawing method

      1.2.1 -1ml frozen cells of HepG2 were taken in pre-warmed 10ml of thaw/culture medium for centrifuging
      1.2.2 -Seed the cells at 2 million per T-75 flask with thaw/culture medium

   1.3. Propagation method

      1.3.1 -Detach the cells from the flask using 0.25% Trypsin
      1.3.2 -The cells are re-seeded in T-225 flask at 3-4 million

2. Assay Protocol

   2.1 -Spin down the cells after rinsing the cells with DPBS and trypsinizing
   2.2 -Resuspend the pellet with thaw/culture medium
   2.3 -Dispense cells in 55 plates of black-clear bottom 1536 well plate at 2000/well/5uL through 8 tip Multidrop plate dispenser
   2.4 -Incubate at 37C for 18hrs (overnight)
   2.5 -Transfer 23nL of compounds from the library collections and positive control to the assay plates through Pintool
   2.6 -Incubate at 37C for 1hr
   2.7 -Read the fluorescence intensity through Envision plate reader for Green (Ex/Em-FITC485/535nm), Blue (Ex/Em-405/460nm) and Red (Ex/Em- 540/590nm)

3. Assay Performance

Auto-Fluorescence (HepG2 cells)	Online Validation Triamterene (Blue channel control) (Mean ± SD)	Online Validation Fluorescein (Green channel control) (Mean ± SD)	Online Validation Rose Bengal sodium (Red channel control) (Mean ± SD)
EC50	NA	NA	NA
S/B	28.19 ±3.03	39.53 ±2.57	19.34 ± 1.46
CV (%)	3.26 ±0.62	3.57 ±0.30	4.78 ±1.73
Z’	0.33 ±0.09	0.77 ±0.06	0.65 ±0.07