

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 REFERENCES:

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 \pm SD)	Online Validation Fluorescein (Green channel control) (Mean \pm SD)	Online Validation Rose Bengal sodium (Red channel control) (Mean \pm SD)
EC50	NA	NA	NA
S/B	28.19 \pm 3.03	39.53 \pm 2.57	19.34 \pm 1.46
CV (%)	3.26 \pm 0.62	3.57 \pm 0.30	4.78 \pm 1.73
Z'	0.33 \pm 0.09	0.77 \pm 0.06	0.65 \pm 0.07