

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

**DOCUMENT:** Auto Fluorescence\_TOX21\_SLP\_Version1.0

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## 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
-DMEM	-Invitrogen	-Invitrogen / 11995
-Fetal Bovine Serum	-Hyclone	-Hyclone / SH30071.03
-Penicillin and Streptomycin	-Invitrogen	-Invitrogen / 15140
-0.05% Trypsin-EDTA	-Invitrogen	-Invitrogen / 25300
-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
-DMEM	-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 HEK293 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 0.05% Trypsin

1.3.2 -The cells are re-seeded in T-225 flask at 3 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 5hrs

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 16hrs

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 (HEK 293 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	26.17 $\pm$ 2.11	28.84 $\pm$ 0.98	16.29 $\pm$ 1.42
CV (%)	4.04 $\pm$ 0.88	3.13 $\pm$ 0.20	7.08 $\pm$ 2.89
Z'	0.22 $\pm$ 0.07	0.78 $\pm$ 0.03	0.68 $\pm$ 0.05