# 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 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 |
|---|---|---|
| -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) | Online Validation Fluorescein (Green channel control) | Online Validation Rose Bengal sodium (Red channel control) |
|---|---|---|---|

| | (Mean ± SD) | (Mean ± SD) | (Mean ± SD) |
|---|---|---|---|
| EC50 | NA | NA | NA |
| S/B | 26.17 ±2.11 | 28.84 ±0.98 | 16.29 ±1.42 |
| CV (%) | 4.04 ±0.88 | 3.13 ±0.20 | 7.08 ±2.89 |
| Z’ | 0.22 ±0.07 | 0.78 ±0.03 | 0.68 ±0.05 |