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 |