Protocol of ELG1 HEK293 Cell-based Assay for High-throughput Screening

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| **DOCUMENT:** |  | ELG1\_TOX21\_SLP\_Version1.0 |
| **TITLE:** |  | Protocol of ELG1 HEK293 Cell-based Assay for High-throughput Screening |

**ASSAY RFERENCES:**

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| --- | --- | --- | --- | --- | --- | --- |
| Assay Target | Cell Lines | Species | Tissue of Origin | Assay Readout | Assay Provider | Toxicity Pathway |
| ATAD5  (Recombinant) | HEK293 | Human | Embryonic kidney | Luciferase reporter | Dr. Myung | Stress response |

**QUALITY CONTROL PRECAUTIONS:**

1. Maintain cells below 85-90% confluence

2. Feed or passage cells twice a week

**MATERIALS and INSTRUMENTS:**

|  |  |  |
| --- | --- | --- |
| Supplies/Medium/Reagent | Manufacturer | Vender/Catalog Number |
| DMEM with GlutaMAX | Invitrogen | Invitrogen / 10564 |
| Fetal Bovine Serum | Hyclone | Hyclone / SH30071.03 |
| Penicillin/Streptomycin | Invitrogen | Invitrogen / 15140 |
| Recovery Cell culture Freezing Medium | Invitrogen | Invitrogen / 12648 |
| 0.05% Trypsin-EDTA | Invitrogen | Invitrogen / 25300 |
| 1536-well white solid plates | Greiner Bio-One | Greiner Bio-One / 789173-F |
| MULTIDROP COMBI | Thermo Electron Corporation | Thermo Electron Corporation |
| BioRAPTR FRD | Beckman Coulter | Beckman Coulter |
| ViewLux Plate Reader | Perkin Elmer | Perkin Elmer |
| Amplite (TM) Luciferase Reporter Gene Assay Kit (Bright Glow) | AAT Bioquest | AAT Bioquest / 12520 |
| CellTiter-Fluor (TM) Cell Viability Assay System | Promega | Promega / TB371 |
| 5-Fluorouridine (Agonist control compound) | Sigma | Sigma/F5130 |

**PROCEDURE:**

1. Cell handling:

1.1. Media Required:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Component | Growth Medium | Assay Medium | Thaw Medium | Freezing Medium |
| DMEM with GlutaMAX | 90% | 90% | 90% | - |
| Fetal Bovine Serum | 10% | 10% | 10% | - |
| Penicillin & Streptomycin | 100U/ml & 100ug/ml | 100U/ml & 100ug/ml | 100U/ml & 100ug/ml | - |
| Recovery Cell culture Freezing Medium | - | - | - | 100% |

1.2. Thawing method

1.2.1 Thaw a vial of cells in 9ml of pre-warmed medium and seed them in T75 flask at 2 million cells

1.3. Propagation method

1.3.1 Trypsinize cells from the flask and centrifuge

1.3.2 Add culture medium to the pellet and passage at 3-4 million per T-225 flask

2. Assay Protocol

2.1 Harvest and resuspend cells in culture/assay medium

2.2 Dispense 2000 cells/5uL/well (for agonist mode) into 1536-well tissue treated white/solid bottom plates

2.3 Incubate the plates for 5hrs at 37C and 5% CO2

2.4 Transfer 23nL of compounds from the library collection and positive control to the assay plates through Pintool

2.5 Incubate the plates for 15.5hrs at 37C and 5% CO2

2.6 Add 1ul of CellTiter-Fluor (TM) Cell Viability reagent (5uL of AFC substrate added in 5ml of Buffer)

2.7 Incubate the plates at room temperature for 30min

2.8 Measure fluorescence by ViewLux plate reader

2.9 Add 4ul of Amplite Luciferase (Bright Glow) reagent

2.10 Incubate the plates at room temperature for 30min

2.11 Measure luminescence by ViewLux plate reader

3. Assay Performance

|  |  |  |
| --- | --- | --- |
| **ATAD5**  **(5-Fluorouridine; Agonist control)** | **Online Validation**  **Agonist**  **(Mean ± SD)** | **Online Validation**  **Viability**  **(Mean ± SD)** |
| EC50 | 1.80 ± 0.18 μM  (n = 27) | NA |
| S/B | 7.08 ± 0.19 | 3.47 ± 0.09 |
| CV (%) ⃰ | 10.66 ± 1.39  (n = 18) | 8.37 ± 0.88  (n = 18) |
| Z’ | 0.81 ± 0.03 | 0.81 ± 0.03 |

⃰ CV values shown represent average of DMSO plates and low concentration plates only.