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

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ASSAY RFERENCES:

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.