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

DOCUMENT: HEK293_WT_TOX21_SLP

TITLE: Protocol of WT HEK293 Cell-based Ca^{2+} Assay for High-throughput Screening

ASSAY RFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
Non-specific Ca++ signaling	HEK293	Human	Embryonic kidney	Calcium dye	Codex Biosolutions	GPCR/Ca++ signaling

QUALITY CONTROL PRECAUTIONS:

1. Maintain cells below 70-80% confluence

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
DMEM	Gibco	Gibco/ 11995-065
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 black clear plates	Greiner Bio-One	Greiner Bio-One / 789092-F
MULTIDROP COMBI	Thermo Electron Corporation	Thermo Electron Corporation
BioRAPTR FRD	Beckman Coulter	Beckman Coulter
FDSS 7000EX kinetic plate reader	Hamamatsu	Hamamatsu
Screen Quest™ Calbryte-520 Probenecid-Free and Wash-Free Calcium Assay Kit	AAT Bioquest	AAT Bioquest / 36319
ATP (Synonyms: Adenosine 5'-triphosphate)	MedChemExpress	MedChemExpress / HY-B2176

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 & 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 Place 9 mL of pre-warmed growth medium into a 15 mL of conical tub.
   1.2.2 Remove the vial of cells to be thawed from liquid nitrogen and thaw rapidly by placing at 37°C in a water bath with gentle agitation for 1-2 minutes.
   1.2.3 Decontaminate the vial by wiping with 70% ethanol before opening in a biological safety cabinet.
   1.2.4 Transfer the vial contents drop-wise into 9 mL of growth medium in a sterile 15-mL conical tub.
   1.2.5 Centrifuge cells at 900 rpm for 4 minutes and resuspend in thaw medium.
   1.2.6 Transfer contents to the T75 tissue culture flask containing Growth Medium and place flask in a humidified 37°C/5% CO_2 incubator.

   1.3. Propagation method

   1.3.1 Aspirate medium, rinse once in DPBS, add 0.05% Trypsin/EDTA and swirl to coat the cell evenly.
   1.3.2 Add an equal volume of Growth Medium to inactivate Trypsin after 2-3 minutes incubation at 37°C.
   1.3.3 Centrifuge cells at 900 rpm for 4 minutes and resuspend in growth medium.
   1.3.4 Cell should be passage or fed at least twice a week.

2. Assay Protocol

   2.1 Harvest and resuspend cells in culture/assay medium
   2.2 Dispense 1500 cells/4 uL/well (for agonist mode) into 1536-well tissue treated black/clear bottom plates using a Multi-drop dispenser.
   2.3 After the cells were incubated at 37°C overnight, add 4 µL of Loading Dye to each well.
   2.4 Incubate the plates for 1 h at 37°C.
   2.5 Incubate the plates for 15 min at room temperature.
   2.6 Add 23 nL of compounds dissolved in DMSO, positive controls or DMSO by a PinTool.
   2.7 Read the fluorescence intensity in FDSS 7000EX kinetic plate reader with a filter set of Ex/Em=480/540 for 5 min at 1 sec intervals

3. Assay Performance

HEK293 WT (ATP; Agonist control)	Screening Agonist (Mean ± SD)
EC50	92.16 ± 33.9 µM (n = 387)
S/B	14.13 ± 2.81
CV (%)*	9.576 ± 1.75 (n = 392)
Z’	0.58 ± 0.08