Protocol of Cell-based hERG Potassium Ion Channel Assay for High-throughput Screening

DOCUMENT: hERG-U2OS_TOX21_SLP_Version1.0

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ASSAY RFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
hERG	U2OS	Human	Osteosarcoma	Thallium influx Fluorecsnce	Codex	hERG potassium ion channel

QUALITY CONTROL PRECAUTIONS:

1. Handle the 1536-well, black-wall, clear-bottom assay plate by the sides; do not touch the clear bottom of the assay plate.

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
DMEM with Glutamin	Invitrogen	10566
Fetal bovine serum	Invitrogen	26140
Nonessential amino acids (NEAA)	Invitrogen	11140
Penicillin/Streptomycin	Invitrogen	15140
0.25% Trypsin/EDTA	Invitrogen	25300
Puromycin	Invitrogen	
DMSO	Sigma	
Recovery Cell Culture	Invitrogen	12648
FDSS 7700EX kinetic plate reader	Hamamatsu	
Black-wall, clear-bottom, 1536-well assay plate	Greiner Bio-One	789092F
FluxOR II green potassium ion channel assay kit	Thermo	F20016

PROCEDURE:

1. Cell handling:
1.1 Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
DMEM with Glutamin	89%		90%	
FBS	10%		10%	
NEAA	1%			
Penicillin/Streptomycin	50 U/mL/50 µg/mL		50 U/mL/50 µg/mL	
Puromycin	1 µg/mL			
Recovery Cell Culture				100%

1.2 Thawing method

1.2.1 Place 14 mL of pre-warmed thaw medium into a 15 mL of conical tub.
1.2.2 Remove the vial of cells 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 cells into 14 mL of thaw medium in a sterile 15 mL conical tube.
1.2.5 Centrifuge cells at 900 rpm for 4 minutes and resuspend in thaw medium.
1.2.6 Transfer cells to the T225 tissue culture flask and place flask in a humidified 37°C/5% CO2 incubator.
1.2.7 Switch to growth medium at first passage.

1.3 Propagation method

1.3.1 Aspirate medium, rinse once in DPBS, add 0.25% 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 cells from culture and resuspend the cells in culture medium.
2.2 Dispense 1000 cells/3 µL/well into 1536-well, black/clear/bottom assay plate using a Multidrop dispenser.
2.3 After the cells were incubated at 37°C and 5% CO2 for 16 hours, 3uL of Loading Buffer was added to each well
2.4 Incubate the plates in at RT in the dark for 1h
2.5 23 nL of compounds dissolved in DMSO, positive controls or DMSO were transferred to the assay plate by a PinTool resulting in a 217-fold dilution
2.6 Incubate the assay plates for 10 min at RT.
2.7 Add 1.5 µL of Stimulation Buffer

2.8 Following adding Stimulation Buffer, fluorescence intensity was continuously measured for 2 min at 1 second interval in the FDSS 700EX kinetic plate reader.

3. Assay performance:

hERG-U2SO	Online Validation (Mean ± SD) (n=27)	Online Validation Viability (Mean ± SD) (n=27)
EC50 (uM)	0.17 ± 0.03	n/a
S/B	4.71 ± 0.21	n/a
CV (%)	3.77 ± 0.59	n/a
Z’	0.85 ± 0.03	n/a