

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 REFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
hERG	U2OS	Human	Osteosarcoma	Thallium influx Fluorescence	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, 3µL 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 μ L 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 \pm SD) (n=27)	Online Validation Viability (Mean \pm SD) (n=27)
EC50 (μ M)	0.17 \pm 0.03	n/a
S/B	4.71 \pm 0.21	n/a
CV (%)	3.77 \pm 0.59	n/a
Z'	0.85 \pm 0.03	n/a