Protocol of ERR Hek293T Cell-based Assay for High-throughput Screening

DOCUMENT: ERR_TOX21_SLP_Version1.0

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ASSAY RFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
ERR regulated genes	ERR Hek293T	Human	Embryonic kidney	Luminescence	NTP	Energy homeostasis

QUALITY CONTROL PRECAUTIONS:

1. The cells should not be grown more than 80-85% confluence
2. Do not leave cells in Trypsin for more than 5 min at RT

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
DMEM, high glucose	Invitrogen	11965-092
HyClone® FBS	Hyclone	SH30071-03
L-glutamine,200mM	Invitrogen	25030-081
Sodium pyruvate solution, 100 mM,	Invitrogen	11360-070
Penicillin/Streptomycin (antibiotic)	Invitrogen	15140
Recovery Cell Culture Freezing Medium	Invitrogen	12648
ONE-Glo Luciferase Assay	Promega	E6051
CellTiter Fluor Cell Viability Assay	Promega	G6080
ViewLux Plate Reader	Perkin Elmer	-
Multidrop	Thermofisher	-
BioRAPTR	Beckman Coulter	-

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
DMEM, high glucose	90%	90%	90%	-
HyClone® FBS	10%	10%	10%	-
Lglutamine,200mM	4mM	4mM	4mM	-

Sodium pyruvate solution, 100 mM	1mM	1mM	1mM	-
Penicillin/Streptomycin (antibiotic)	1%	1%	1%	-
Recovery Cell Culture Freezing Medium	-	-	-	100%

1.2. Thawing method

1.2.1 Place 14 mL of pre-warmed thaw medium into a 15 mL conical tube
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
1.2.3 Mix the entire content of the vial to 14 ml of pre-warmed medium and centrifuge to remove DMSO
1.2.4 Discard the supernatant and reconstitute the pellet using pre-warmed thaw medium
1.2.5 Transfer the cells to T75 flask

1.3. Propagation method

1.3.1 Detach the cells from the flask using Trypsin-EDTA (0.05%)
1.3.2 The cells in growth medium are re-seeded in T225 flask

2. Assay Protocol

2.1 Harvest cells and re-suspend in assay medium and adjust the required cell density
2.2 Dispense 2500 cells/5 µL/well into 1536 well tissue treated white plates using a Multidrop dispenser
2.3 Incubate the plates at a 37ºC, 5% CO2 incubator for 6 h
2.4 Transfer 23nL of compounds and positive control to the assay plate by a pin tool.
2.5 Incubate the assay plates at 37°C, 5% CO2 for 17.5 h
2.6 Add 1 µL of CellTiter-Fluor regent using a BioRAPTR
2.7 Incubate at 37ºC, 5% CO2 for 17.5 h
2.8 Read the fluorescence intensity in ViewLux plate reader
2.6 Add 4uL of OneGlo and incubate the plates at room temperature for 0.5 h
2.7 Read the luminescence intensity in ViewLux plate reader

3. Assay Performance

ERR-agonist **(Genistein)**	Online Validation (Mean ± SD)
EC50 (µM)	4.09±0.48 (n=27)
S/B	1.76±0.05 (n=27)
CV (%)	3.84±0.81 (n=27)
Z’	0.37±0.07 (n=27)

ERR-antagonist **(XCT790)**	Online Validation (Mean ± SD)
IC50 (µM)	4.50±0.47 (n=27)

S/B	3.54±0.17 (n=27)
CV (%)	3.84±0.81 (n=27)
Z’	0.77±0.03 (n=27)

ERR-Viability **(Tetra-Br.)**	Online Validation (Mean ± SD)
IC50 (µM)	N/A
S/B	8.81±0.40 (n=27)
CV (%)	5.09±0.95 (n=27)
Z’	0.79±0.03 (n=27)