Protocol of TRE-GH3 GH3 Cell-based Assay for High-throughput Screening

DOCUMENT: TRE-GH3_TOX21_SLP_Version1.0
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ASSAY RFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
Thyroid receptor: full (Endogenous)	GH3	Rat	Pituitary tumor GH3	Luminescence	Dr. Murk	NR signaling

QUALITY CONTROL PRECAUTIONS:

1. -

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
-DMEM:F12	-Invitrogen	-Gibco 10565
-Fetal Bovine Serum	Hyclone	Hyclone, SH30071.03
Pen/Strep	Invitrogen	Invitrogen, 15140
insulin	sigma	sigma, I6634
ethanolamine	sigma	sigma, E0135
sodium selenite	sigma	sigma, S5261
-human apo-Transferrin	sigma	sigma, T2036
Bovine Serum Albumin	sigma	sigma, A9647
TrypLE Express	Invitrogen	Invitrogen, 12605
-PBS without calcium and magnesium	invitrogen	-invitrogen, 14190
Recovery cell culture freezing medium	invitrogen	invitrogen, 12648
-centrifuge	sorvall legend XTR	Thermo Fisher Science 75004520
BioRAPTR, Microfluidic workstation	beckmen	-
-Pintool	Kalypsys	-
white, tc, sterile 1536-well assay plates	Greiner Bio-One	-Greiner, 789173-F

-Viewlux plate reader	PerkinElmer	-
T3 (Agonist control compound)	Calbiochem	Calbiochem, 642511
-DMSO	AMRESCO	-KD medical, RGE-3070
-One-Glo	Promega	Promega, E6120

PROCEDURE:

1. Cell handling:
   1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
-Recovery Cell Culture Medium	-	-	-	100%
- DMEM:F12	90%	100%	90%	-
-fetal bovine serum	10%	-	10%	-
-Pen/strep	100U/mL-100ug/mL	-	100U/mL-100ug/mL	-
-insulin	-	10ug/mL	-	-
-ethanolamine	-	10uM	-	-
-sodium selenite	-	10ng/mL	-	-
-human apo-Transferrin	-	10ug/mL	-	-
-bovine serum albumin	-	500ug/mL	-	-
-	-	-	-	-

   1.2. Thawing method

   1.2.1 -Place 14 mL of pre-warmed thaw medium into a T75 flask
   1.2.2 -Remove the vial of cells to be thawed from liquid nitrogen and thaw rapidly by placing at 37C in a water bath with gentle agitation for 1-2 minutes. Do not submerge vial in water.
   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 10 mL of Thaw Medium in a sterile 15-mL conical tube
   1.2.5 -Centrifuge cells at 1000 rpm for 4 mins
   1.2.6 -Transfer contents to the T75 tissue culture flask containing Thaw Medium and place flask in a humidified 37C/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 TrypLE Express(3 mL for a T75 flask and 5 mL for a T175 flask and 7.5 mL for T225 flask) and swirl to coat the cell evenly.
1.3.2 -Add an equal volume of Growth Medium to inactivate Trypsin after 2-3 mins incubation at 37C.
1.3.3 -Centrifuge cells at 1000 rpm for 4 mins 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 in Growth Medium and resuspend in assay medium
2.2 -Dispense 1500 cells/4μL/well into 1536-well tissue treated white solid plates using a BioRAPTR dispenser.
2.3 -After the cells were incubated at 37C for 4 hrs, 23 nL of compounds dissolved in DMSO, positive controls or DMSO were transferred to the assay plate by a PinTool
2.4 -Add 1uL of T3 or buffer control using BioRaptr
2.5 -Incubate the plates for 24 hrs at 37C.
2.6 -Add 5μL of One-Glo to each well using a BioRAPTR dispenser and incubate the plate at room temperature for 30 mins.
2.7 -Measure luminescence using Viewlux

3. Assay Performance

GH3-TRE (Antagonist control not available)	**Online Validation Antagonist (Mean ± SD)**
IC50	N/A
S/B	4.39 ± 1.57
CV (%)*	12.17 ± 1.96 (n = 18)
Z’	0.39 ± 0.09

*CV values shown represent average of DMSO plates and low concentration plates only.