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
Penicillin/Streptomycin (antibiotic)	-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
-Phosphate-buffered saline 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%	-
-Penicillin-Streptomycin	-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 tub
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/5 µL/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 resulting in a 217-fold dilution.
2.4 Incubate the plates for 24 hrs at 37C.
2.5 Add 5 µL of One-Glo to each well using a BioRAPTR dispenser and incubate the plate at room temperature for 30 mins.
2.6 -Measure luminescence using Viewlux

3. Assay Performance

GH3-TRE (T3; Agonist control)	**Online Validation Agonist (Mean ± SD)**
EC50	0.67 ± 0.20 nM (n = 27)
S/B	6.64 ± 0.28
CV (%)*	8.75 ± 1.80 (n = 18)
Z’	0.77 ± 0.04

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