

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

DOCUMENT: TRE-GH3_TOX21_SLP_Version1.0

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

ASSAY REFERENCES:

| 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 \pm SD) |
|---|--|
| IC50 | N/A |
| S/B | 4.39 \pm 1.57 |
| CV (%) * | 12.17 \pm 1.96 (n = 18) |
| Z' | 0.39 \pm 0.09 |

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