Protocol of ROR-gamma CHO Cell-based Assay for High-throughput Screening

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| **DOCUMENT:** |  | ROR-gamma\_TOX21\_SLP\_Version1.0 |
| **TITLE:** |  | Protocol of ROR-gamma CHO Cell-based Assay for High-throughput Screening |

**ASSAY RFERENCES:**

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| --- | --- | --- | --- | --- | --- | --- |
| Assay Target | Cell Lines | Species | Tissue of Origin | Assay Readout | Assay Provider | Toxicity Pathway |
| Retinoid-related Orphan Receptor gamma | CHO | Human | Chinese Hamster Ovary | Luciferase reporter | Dr. Jetten | ROR pathway |

**QUALITY CONTROL PRECAUTIONS:**

1. -Maintain cells below 85-90% confluence.

**MATERIALS and INSTRUMENTS:**

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| Supplies/Medium/Reagent | Manufacturer | Vender/Catalog Number |
| -F12 medium | -Invitrogen | -Invitrogen/11765 |
| -FBS approved to use with Tet-on system | -Clontech | -Clontech/631101 |
| -Penicillin & Streptomycin | -Invitrogen | -Invitrogen/15140 |
| -Recovery Cell culture Freezing Medium | -Invitrogen | -Invitrogen/12648 |
| -0.05% Trypsin-EDTA | -Invitrogen | -Invitrogen/25300 |
| -TO901317 (Antagonist control compound) | -Sigma | -Sigma/T2320 |
| -Doxycycline Hyclate | -Sigma | -Sigma/D9891 |
| -1536-well white solid plates | -Greiner Bio-One | -Greiner Bio-One / 789173-F |
| -MULTIDROP COMBI | -Thermo Electron Corporation | -Thermo Electron Corporation |
| -BioRAPTR FRD | -Beckman Coulter | -Beckman Coulter |
| -ViewLux Plate Reader | -Perkin Elmer | -Perkin Elmer |
| -ONE-Glo(TM) Luciferase Assay System | -Promega | -Promega / E6120 |

**PROCEDURE:**

1. Cell handling:

1.1. Media Required:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Component | Growth Medium | Assay Medium | Thaw Medium | Freezing Medium |
| -F12 medium | -90% | -90% | -90% | - |
| -FBS approved to use with Tet-on system | -10% | -10% | -10% | - |
| -Penicillin & Streptomycin | -100U/ml & 100ug/ml | -100U/ml & 100ug/ml | -100U/ml & 100ug/ml | - |
| -Recovery Cell culture Freezing Medium | - | - | - | -100% |

1.2. Thawing method

1.2.1 -Thaw a vial of cells in 9ml of pre-warmed thaw medium and then centrifuge

1.2.2 -Resuspend the pellet with the thaw medium and seed at 2 million cells per T-75 flask

1.3. Propagation method

1.3.1 -Trypsinize cells from the culturing flask and centrifuge and then resuspend cells in culture medium

1.3.2 -Passage cells at 2-3 million per T-225 flask

2. Assay Protocol

2.1 -Trypsinize cells from the culturing flask and centrifuge and then resuspend cells in assay medium at a density of 0.25 X 10^6 cells/mL

2.2 -Dispense 1000 cells/4uL/well into 1536-well tissue treated white/solid bottom plates using a 8 tip dispenser (Multidrop)

2.3 -Incubate the plates for 5hrs at 37C and 5% CO2

2.4 -Transfer 23nL of compounds from the library collection (0.59nM to 92uM) and positive control through Pintool

2.5 -Incubate the plates for 2hrs at 37C and 5% CO2

2.6 -Add 1ul of 1.0uM (final concentration) Doxycycline Hyclate in assay buffer using single tip dispense (Bioraptr)

2.7 -Incubate the plates for 16hrs at 37C and 5% CO2

2.8 -Then add 5ul of ONE-Glo(TM) Luciferase reagent using a single tip dispense (Bioraptr)

2.9 -Incubate the plates at room temperature for 30min

2.10 -Measure luminescence (exposure time = 5 sec) by ViewLux plate reader

3. Assay Performance

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| --- | --- |
| **ROR**γ  **(TO901317; Antagonist control)** | **Online Validation**  **Antagonist**  **(Mean** ± **SD)** |
| IC50 | 6.02 ± 0.59 μM  (n = 27) |
| S/B | 77.52 ± 10.49 |
| CV (%) ⃰ | 11.73 ± 0.41  (n = 18) |
| Z’ | 0.64 ± 0.04 |

⃰ CV values shown represent average of all plates excluding top 3 compound concentration plates.