

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

DOCUMENT: ROR-gamma_TOX21_SLP_Version1.0

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

ASSAY REFERENCES:

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:

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 0.25×10^6 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

ROR_γ (TO901317; Antagonist control)	Online Validation Antagonist (Mean ± SD)
IC ₅₀	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.