

Protocol of Aromatase Breast cancer cell line (MCF-7 aro) Cell-based Assay for High-throughput Screening

DOCUMENT: Aromatase_TOX21_SLP_Version1.0

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ASSAY REFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
Aromatase Inhibition (Endogenous)	Breast cancer cell line (MCF-7 aro)	Human	Mammary gland, breast	ERE-luciferase reporter expression	Shiuan Chen	Aromatase/ER/ ERR pathways

QUALITY CONTROL PRECAUTIONS:

1. -Maintain cell culture below 85% confluence
2. -The culture medium was replaced to assay medium (containing 10% Charcoal stripped FBS) two days prior to the assay

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
-MEM/EBSS medium	-Hyclone	-Hyclone / SH 30024.01
-FBS, Heat Inactivated, USDA approved	-Invitrogen	-Invitrogen / 10438
-Charcoal stripped FBS	-Invitrogen	-Invitrogen / 12676
-Sodium pyruvate	-Invitrogen	-Invitrogen / 11360
-Penicillin & Streptomycin	-Invitrogen	-Invitrogen / 15140
-Hygromycin	-Invitrogen	-Invitrogen / 10687
-G418 (Geneticin)	-Invitrogen	-Invitrogen / 10131
-L-Glutamine	-Invitrogen	-Invitrogen / 25030
-Recovery Cell culture Freezing Medium	-Invitrogen	-Invitrogen / 12648
-MEM 1x with Earle's salts, without L-glutamine and phenol red Medium	-Mediatech	-Mediatech / 17-305-CV
-0.25% Trypsin-EDTA	-Invitrogen	-Invitrogen / 25200
-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
-CellTiter-Fluor (TM) Cell Viability Assay	-Promega	-Promega / G6082
-Letrozole (Antagonist control compound)	-Tocris	-Tocris/4382
-Testosterone (Agonist control compound)	-Sigma	-Sigma/T1500

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
-MEM/EBSS medium	-90%	-	-90%	-
-FBS, Heat Inactivated, USDA approved	-10%	-	-10%	-
-MEM 1x with Earle's salts, without L-glutamine and phenol red Medium	-	-90%	-	-
-Charcoal/dextran treated FBS	-	-10%	-	-
-Sodium pyruvate	-1mM	-1mM	-1mM	-
-Hygromycin	-20ug/ml	-	-	-
-G418 (Geneticin)	-50ug/ml	-	-	-
-L-Glutamine	-	-2mM	-	-
-Recovery Cell culture Freezing Medium	-	-	-	-100%
-Penicillin & Streptomycin	-100U/ml & 100ug/ml	-100U/ml & 100ug/ml	-100U/ml & 100ug/ml	-

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 6-7 million per T-225 flask

2. Assay Protocol

2.1 -Trypsinize cells from the culturing flask (medium changed to assay medium two days prior) and centrifuge and then resuspend cells in assay medium

2.2 -Dispense 1500 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 -Add 1ul of assay buffer with or without 0.5nM (final concentration) Testosterone using two tips of a dispenser (Bioraptr)

2.6 -Incubate the plates for 23.50hrs at 37C and 5% CO2

- 2.7 -Add 1ul of CellTiter-Fluor (TM) Cell Viability Assay reagent using a single tip dispenser (Bioraptr)
 2.8 -Incubate the plates at 37C for 30min
 2.9 -Measure fluorescence by ViewLux plate reader
 2.10 -Then add 4ul of ONE-Glo(TM) Luciferase reagent using a single tip dispenser (Bioraptr)
 2.11 -Incubate the plates at room temperature for 30min
 2.12 -Measure luminescence (exposure time = 15 sec) by ViewLux plate reader

3. Assay Performance

Aromatase (Letrozole; Antagonist control)	Online Validation Antagonist (Mean \pm SD)	Online Validation Viability (Mean \pm SD)
IC50	9.44 \pm 1.4 nM (n = 27)	NA
S/B	5.98 \pm 0.18	3.72 \pm 0.09
CV (%) *	4.43 \pm 0.99 (n = 18)	6.38 \pm 0.42 (n = 18)
Z'	0.79 \pm 0.07	0.80 \pm 0.03

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