Protocol of AR-MDA MDA-MB-453 Cell-based Assay for High-throughput Screening

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ASSAY RFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
Androgen receptor	MDA-MB-453	Human	Mammary gland, breast	Luminescence	ATCC	NR signaling

QUALITY CONTROL PRECAUTIONS:

1. -Maintain cell culture below 85-90% confluence
2. -Cell culturing and assay culture doesnot require CO2

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
-Leibovitz's L-15 Medium	-ATCC	-ATCC / 30-2008
-Fetal Bovine Serum	-Hyclone	-Hyclone / SH30071.03
-Penicillin/Streptomycin	-Invitrogen	-Invitrogen / 15140
-Recovery Cell culutre Freezing Medium	-Invitrogen	-Invitrogen / 12648
-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
-R1881 (Agonist Reference Compound)	-Perkin Elmer	-Perkin Elmer / NLP005005MG
-Nilutamide (Antagonist Reference Compound)	-Sigma	-Sigma / N8534
-Tetraoctyl ammonium bromide (Viability Reference Compound)	-Sigma	-Sigma / 294136

PROCEDURE:

1. Cell handling:
   1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
-Leibovitz's L-15 Medium	-100%	-100%	-100%	-
-Fetal Bovine Serum	-10%	-10%	-10%	-
-Penicillin & Streptomycin	-100U/ml & 100ug/ml	-100U/ml & 100ug/ml	-100U/ml & 100ug/ml	-
-Recovery Cell culutre Freezing Medium	-	-	-	-100%

   1.2. Thawing method

      1.2.1 -Thaw a vial of cells in 9ml of pre-warmed thaw/culture medium and then centrifuge
      1.2.2 -Resuspend the pellet with the thaw/culutre 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 and centrifuge and then resuspend cells in culture/assay medium
   2.2 -Dispense 3000 cells/5uL/well (for agonist mode) into 1536-well tissue treated white/solid bottom plates using a 8 tip dispenser (Multidrop)
   2.3 -Incubate the assay plates for 5-6hrs at 37C and 0% CO2
   2.4 -Transfer 23nL of compounds from the library collection and positive control/DMSO into the assay plates through Pintool
   2.5 -Compound transfer was followed by the addition of 1uL of 0.5nM (final concentration) R1881 or assay buffer using 2 tips of a dispenser (BioRAPTR FRD)

2.6 -Incubate the assay plates for 16hrs at 37C and 0% CO2
2.7 -After 15hrs of incubation at 37C and 0%CO2, 1ul of CellTiter-Fluor reagent was added using a single tip of a dispenser (BioRAPTR FRD)
2.8 -Incubate the assay plates at 37C and 0%CO2 for 1hr
2.9 -Read fluorescence using ViewLux plate reader
2.10 -Then followed by the addition of 4ul of ONE-Glo(TM) Luciferase reagent using a single tip of a dispenser (BioRAPTR FRD)
2.11 -Incubate the assay plates at room temperature for 30min
2.12 -Read Luminescence using ViewLux plate reader