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

DOCUMENT: AR-MDA_TOX21_SLP_Version1.0

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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 doesn't 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 culture Freezing Medium	-Invitrogen	-Invitrogen / 12648
-0.05% Trypsin-EDTA	-Invitrogen	-Invitrogen / 25300
-1536-well white solid plates	-Corning	-Corning / 7464
-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 positive control compound)	-Perkin Elmer	-Perkin Elmer/NLP005005MG
-Nilutamide (Antagonist positive control compound)	-Sigma Aldrich	-Sigma Aldrich/N8534
-Tetraoctyl ammonium bromide (Viability positive control compound)	-Sigma Aldrich	-Sigma Aldrich/294136

PROCEDURE:

1. Cell handling:

AR-MDA-kb2 Nilutamide added	Online Validation Agonist (Mean ± SD)	Online Validation Viability (Mean ± SD)
EC50 Nilutamide-free R1881	0.29 ± 0.04 nM (n = 27)	NA
EC50 R1881 with 3.0uM Nilutamide	8.16 ± 1.05 nM (n = 26)	NA
S/B	9.07 ± 0.37	10.23 ± 0.25
CV (%)*	7.57 ± 0.89 (n = 24)	6.21 ± 0.38 (n = 24)
Z’	0.76 ± 0.05	0.81 ± 0.02

1.1. Media Required:

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 –Re-suspend the pellet with the thaw/culture 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 re-suspend 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 re-suspend cells in culture/assay medium.
2.2 -Dispense 3000 cells/4uL/well into 1536-well tissue treated white/solid bottom plates using an 8 tip dispenser (Multidrop).
2.3 -Incubate the assay plates for 5hrs at 37C and 0% CO2.
2.4 -First 1uL of 3.0uM (final concentration) Nilutamide or assay buffer was added using two separate tips of a dispenser (BioRAPTR).
2.5 -Then 23nL of compounds were transferred from the library collection into 5-48 columns and positive control into 1-4 columns using a Pintool station.
2.6 -Incubate the assay plates for 16hrs at 37C and 0% CO2.
2.7 -After 15hrs of incubation, 1ul of CellTiter-Fluor(TM) Cell Viability Assay reagent was added using a single tip of a dispenser (BioRAPTR).
2.8 -Incubate the assay plates at 37C and 0% CO2 for 1hr.
2.9 -Measure fluorescence signal by ViewLux plate reader (Exposure time = 1sec).
2.10 -Add 4ul of ONE-Glo(TM) Luciferase reagent using a single tip of a dispenser (BioRAPTR).
2.11 -Incubate the assay plates at room temperature for 30min.
2.12 -Measure luminescence signal by ViewLux plate reader (Exposure time = 90sec).

3. Assay Performance

* CV values shown represent average of all assay plates excluding the top concentration plates