

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

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ASSAY REFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
Androgen receptor (Endogenous)	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	-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	-Promega	-Promega / G6082

Assay		
Cyproterone acetate (Antagonist control compound)	-Sigma Aldrich	-Sigma Aldrich/C3412

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 culture 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/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 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 plates for 5hrs at 37C and 0% CO2

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

2.5 -Incubate the plates for 15.30hrs at 37C and 0% CO2

2.6 -Add 1ul of CellTiter-Fluor (TM) Cell Viability Assay reagent using a single tip dispenser (Bioraptr)

2.7 -Incubate the plates at room temperature or 37C for 30min

2.8 -Measure fluorescence by ViewLux plate reader

2.9 -Then add 4ul of ONE-Glo(TM) Luciferase reagent using a single tip dispenser (Bioraptr)

2.10 -Incubate the plates at room temperature for 30min

2.11 -Measure luminescence by ViewLux plate reader

3. Assay Performance

AR-MDA-kb2 (Cyproterone acetate; Antagonist control)	Online Validation Antagonist (Mean ± SD)	Online Validation Viability (Mean ± SD)
IC50	0.15 ± 0.87 µM (n = 27)	NA
S/B	7.66 ± 0.40	1.25 ± 0.02
CV (%)*	10.15 ± 2.03 (n = 18)	8.74 ± 2.63 (n = 18)
Z'	0.48 ± 0.08	0.23 ± 0.10

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