

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

DOCUMENT: AR-MDA_TOX21_SLP_Version1.0

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

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
R1881 or Methyltrienolone (Agonist control compound)	-Perkin Elmer	- Perkin Elmer/ NLP005005MG

PROCEDURE:

1. Cell handling:
 - 1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
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-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 16hrs at 37C and 0% CO₂

2.6 -Add 5ul of ONE-Glo(TM) Luciferase reagent using a single tip dispenser (Bioraptr)

2.7 -Incubate the plates at room temperature for 30min

2.8 -Measure luminescence by ViewLux plate reader

3. Assay Performance

AR-MDA-kb2 (R1881; Agonist control)	Online Validation Agonist (Mean \pm SD)
EC50	0.10 \pm 0.02 nM (n = 27)
S/B	6.28 \pm 0.35
CV (%) [*]	15.89 \pm 5.84 (n = 18)
Z'	0.40 \pm 0.19

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