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

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| **DOCUMENT:** |  | AR-MDA\_TOX21\_SLP\_Version1.0 |
| **TITLE:** |  | Protocol of AR-MDA MDA-MB-453 Cell-based Assay for High-throughput Screening |

**ASSAY RFERENCES:**

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| --- | --- | --- | --- | --- | --- | --- |
| 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:**

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| --- | --- | --- |
| 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 Assay | -Promega | -Promega / G6082 |
| 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

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| **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.