Protocol of Aromatase Breast cancer cell line (MCF-7 aro) Cell-based Assay for High-throughput Screening

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| **DOCUMENT:** |  | Aromatase\_TOX21\_SLP\_Version1.0 |
| **TITLE:** |  | Protocol of Aromatase Breast cancer cell line (MCF-7 aro) 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 |
| Aromatase Inhibition  (Endogenous) | Breast cancer cell line (MCF-7 aro) | Human | Mammary gland, breast | ERE-luciferase reporter expression | Shiuan Chen | Aromatase/ ER/ ERR pathways |

**QUALITY CONTROL PRECAUTIONS:**

1. -Maintain cell culture below 85% confluence

2. -The culture medium was replaced to assay medium (containing 10% Charcoal stripped FBS) two days prior to the assay

**MATERIALS and INSTRUMENTS:**

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| Supplies/Medium/Reagent | Manufacturer | Vender/Catalog Number |
| -MEM/EBSS medium | -Hyclone | -Hyclone / SH 30024.01 |
| -FBS, Heat Inactivated, USDA approved | -Invitrogen | -Invitrogen / 10438 |
| -Charcoal stripped FBS | -Invitrogen | -Invitrogen / 12676 |
| -Sodium pyruvate | -Invitrogen | -Invitrogen / 11360 |
| -Penicillin & Streptomycin | -Invitrogen | -Invitrogen / 15140 |
| -Hygromycin | -Invitrogen | -Invitrogen / 10687 |
| -G418 (Geneticin) | -Invitrogen | -Invitrogen / 10131 |
| -L-Glutamine | -Invitrogen | -Invitrogen / 25030 |
| -Recovery Cell culture Freezing Medium | -Invitrogen | -Invitrogen / 12648 |
| -MEM 1x with Earle’s salts, without L-glutamine and phenol red Medium | -Mediatech | -Mediatech / 17-305-CV |
| -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 |
| -Letrozole (Antagonist control compound) | -Tocris | -Tocris/4382 |
| -Testosterone (Agonist control compound) | -Sigma | -Sigma/T1500 |

**PROCEDURE:**

1. Cell handling:

1.1. Media Required:

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| --- | --- | --- | --- | --- |
| Component | Growth Medium | Assay Medium | Thaw Medium | Freezing Medium |
| -MEM/EBSS medium | -90% | - | -90% | - |
| -FBS, Heat Inactivated, USDA approved | -10% | - | -10% | - |
| -MEM 1x with Earle's salts, without L-glutamine and phenol red Medium | - | -90% | - | - |
| -Charcoal/dextran treated FBS | - | -10% | - | - |
| -Sodium pyruvate | -1mM | -1mM | -1mM | - |
| -Hygromycin | -20ug/ml | - | - | - |
| -G418 (Geneticin) | -50ug/ml | - | - | - |
| -L-Glutamine | - | -2mM | - | - |
| -Recovery Cell culture Freezing Medium | - | - | - | -100% |
| -Penicillin & Streptomycin | -100U/ml & 100ug/ml | -100U/ml & 100ug/ml | -100U/ml & 100ug/ml | - |

1.2. Thawing method

1.2.1 -Thaw a vial of cells in 9ml of pre-warmed thaw medium and then centrifuge

1.2.2 -Resuspend the pellet with the thaw 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 (medium changed to assay medium two days prior) and centrifuge and then resuspend cells in assay medium

2.2 -Dispense 1500 cells/4uL/well 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 5% CO2

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

2.5 -Add 1ul of assay buffer with or without 0.5nM (final concentration) Testosterone using two tips of a dispenser (Bioraptr)

2.6 -Incubate the plates for 23.50hrs at 37C and 5% CO2

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

2.8 -Incubate the plates at 37C for 30min

2.9 -Measure fluorescence by ViewLux plate reader

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

2.11 -Incubate the plates at room temperature for 30min

2.12 -Measure luminescence (exposure time = 15 sec) by ViewLux plate reader

3. Assay Performance

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| **Aromatase**  **(Letrozole; Antagonist control)** | **Online Validation**  **Antagonist**  **(Mean ± SD)** | **Online Validation**  **Viability**  **(Mean ± SD)** |
| IC50 | 9.44 ± 1.4 nM  (n = 27) | NA |
| S/B | 5.98 ± 0.18 | 3.72 ± 0.09 |
| CV (%) ⃰ | 4.43 ± 0.99  (n = 18) | 6.38 ± 0.42  (n = 18) |
| Z’ | 0.79 ± 0.07 | 0.80 ± 0.03 |

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