Protocol of ER-alpha-BG1 BG1 Cell-based Assay for High-throughput Screening

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| **DOCUMENT:** |  | ER-alpha-BG1\_TOX21\_SLP\_Version1.0 |
| **TITLE:** |  | Protocol of ER-alpha-BG1 BG1 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 |
| Estrogen receptor alpha: full  (Endogenous) | BG1 | Human | Ovarian adenocarcinoma | Luciferase reporter | UC Davis | NR signaling |

**QUALITY CONTROL PRECAUTIONS:**

1. -Maintain cells below 85-90% confluence in culture medium

2. -For assay purpose, the cells should be cultured in assay medium with 10% charcoal stripped FBS for 5 days with alternate day medium changed to fresh medium

3. -Especially while in assay culture, the cells should not reach more than 85% confluence as they would become harder to detach if they reach over confluence

**MATERIALS and INSTRUMENTS:**

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| --- | --- | --- |
| Supplies/Medium/Reagent | Manufacturer | Vender/Catalog Number |
| -MEM α medium | -Invitrogen | -Invitrogen, 12561 |
| -10% Premium Fetal Bovine Serum | -Atlanta Biologicals | -Atlanta Biologicals, S11150 |
| -Penicillin/Streptomycin | -Invitrogen | -Invitrogen, 15140 |
| -400mg/l G418 (Geneticin) | -Invitrogen | -Invitrogen, 10131 |
| -DMEM phenol red free - low glucose medium | -Invitrogen | -Sigma, D5921 |
| -Charcoal stripped Fetal Bovine Serum | -Invitrogen | -Invitrogen, 12676 |
| -L-Glutamine | -Invitrogen | -Invitrogen, 25030 |
| -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 Luciferase Assay system | -Promega | -Promega / E6130 |
| -Recovery Cell culture Freezing Medium | -Invitrogen | -Invitrogen / 12648 |
| -CellTiter-Fluor Cell Viability Assay | -Promega | -Promega / G6082 |
| 4-Hydroxytamoxifen  (Antagonist control compound) | Sigma | Sigma/H7904 |

**PROCEDURE:**

1. Cell handling:

1.1. Media Required:

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| --- | --- | --- | --- | --- |
| Component | Growth Medium | Assay Medium | Thaw Medium | Freezing Medium |
| -MEM α medium | -90% | - | -90% | - |
| -DMEM phenol red free - low glucose medium | - | -90% | - | - |
| -Premium Fetal Bovine Serum | -10% | - | -10% | - |
| -Charcoal/dextran treated Fetal Bovine Serum | - | -10% | - | - |
| -Penicillin/Streptomycin | -100U/ml & 100ug/ml | -100U/ml & 100ug/ml | -100U/ml & 100ug/ml | - |
| -L-Glutamine | - | -2mM | - | - |
| -G418 (Geneticin) | -400mg/l | - | - | - |
| -Recovery Cell culture Freezing Medium | - | - | - | -100% |

1.2. Thawing method

1.2.1 -Thaw a frozen vial of cells in 9ml of pre-warmed medium and seed them in T175 flask at 2 million cells

1.3. Propagation method

1.3.1 -Trypsinize cells from the flask and centrifuge

1.3.2 -Add culture medium to the pellet and passage at 3-4 million per T-225 flask

2. Assay Protocol

2.1 -Harvest cells from the 5-day culture in assay medium and resuspend cells in assay medium

2.2 -Dispense 4000 cells/5uL/well into 1536-well tissue treated white/solid bottom plates

2.3 -Incubate the plates for 24hrs at 37C and 5% CO2

2.4 -Transfer 23nL of compounds from the library collection and positive control to the assay plates through Pintool

2.5 -Add 1uL of 0.5nM (final) Beta-Estradiol (agonist) or assay buffer

2.6 -Incubate the plates for 21.5hrs at 37C and 5% CO2

2.7 -Add 1ul of CellTiter-Fluor reagent

2.8 -Incubate the plates at 37C for 30min

2.9 -Measure fluorescence by ViewLux plate reader

2.10 -Add 4ul of ONE-Glo reagent

2.11 -Incubate the plates at room temperature for 30min

2.12 -Measure luminescence by ViewLux plate reader

3. Assay Performance

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| **ERα-BG1**  **(4-Hydroxy Tamoxifen;**  **Antagonist control)** | **Online Validation**  **Antagonist**  **(Mean ± SD)** |
| IC50 | 0.04 ± 0.004 μM  (n = 27) |
| S/B | 7.88 ± 0.39 |
| CV (%) ⃰ | 8.27 ± 5.78  (n = 18) |
| Z’ | 0.73 ± 0.10 |

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