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

DOCUMENT: ER-alpha-BG1_TOX21_SLP_Version1.0

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ASSAY RFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
Estrogen receptor alpha: full	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:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
-MEM α medium	-Invitrogen	-Invitrogen/12561
-Premium Fetal Bovine Serum	-Atlanta Biologicals	-Atlanta Biologicals/S11150
-Penicillin/Streptomycin	-Invitrogen	-Invitrogen/15140
-G418 (Geneticin)	-Invitrogen	-Invitrogen/10131
-DMEM phenol red free - low glucose medium	-Invitrogen	-Sigma-Aldrich/D5921
-Charcoal stripped Fetal Bovine Serum	-Invitrogen	-Invitrogen/12676
-L-Glutamine	-Invitrogen	-Invitrogen/25030
-0.25% Trypsin-EDTA	-Invitrogen	-Invitrogen/25200
-Recovery Cell culutre Freezing Medium	-Invitrogen	-Invitrogen / 12648

-MULTIDROP COMBI	-Thermo Electron Corporation	-Thermo Electron Corporation
-BioRAPTR FRD	-Beckman Coulter	-Beckman Coulter
-ViewLux Plate Reader	-Perkin Elmer	-Perkin Elmer
-1536-well white solid plates	-Corning	-Corning/7464
-CellTiter-Fluor(TM) Cell Viability Assay	-Promega	-Promega/G6082
-ONE-Glo(TM) Luciferase Assay System	-Promega	-Promega / E6130
-Beta-Estradiol	-Sigma-Aldrich	-Sigma-Aldrich/E8875
-4-Hydroxy Tamoxifen	-Sigma-Aldrich	-Sigma-Aldrich/H7904
-Tetraoctyl ammonium bromide	-Sigma-Aldrich	-Sigma-Aldrich/294136

PROCEDURE:

1. Cell handling:
    1.1. Media Required:

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 stripped 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 culutre Freezing Medium	-	-	-	-100%

1.2. Thawing method

1.2.1 -Thaw a frozen vial of cells in 9ml of pre-warmed thaw 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 mdeium to the pellet and passage at 5 million per T-225 flask

2. Assay Protocol

2.1 -The frozen vial containing 15.0 million cells of BG1-Luc-4E2 was thawed in a five-layer flask for 3 days in culture and passaged with culture medium at 15.0-20.0 million cells per five-layer flask.
2.2 -After 48hrs of culture in culture medium, the medium is changed to assay medium and culture for an another 5 days prior to the assay with alternate day medium changed to fresh medium.
2.3 -BG1-Luc-4E2 cells (Passage 10) were dispensed at 4000 cells per well in 4uL of assay medium into 1536-well tissue treated white/solid bottom plates using 8 tip of a Multidrop dispenser.
2.4 -The assay plates were incubated at 37C and 5%CO2 for 24hrs.
2.5 -Transferred 23nL of positive control into 1-4 columns and compounds from the library collection into 5-48 columns of the assay plate and using Pintool Station.
2.6 -Then followed by the addition of 1uL of 0.1nM (final concentration) Beta-Estradiol (agonist) or assay buffer using two seperate tips of a Bioraptr dispenser.
2.7 -After compound addition, the assay plates were incubated at 37C and 5%CO2 for 22hrs.
2.8 -For measuring cytotoxicity, after 21hrs of incubation at 37C and 5%CO2, one uL of CellTiter-Fluor (TM) reagent was added using a single tip of a Bioraptr dispenser.
2.9 -The assay plates were incubated at 37C and 5%CO2 for 1hr.
2.10 -The fluorescence was read using ViewLux plate reader. (Exposure time: 1sec)
2.11 -Then 4ul of ONE-Glo Luciferase assay reagent was added using a single tip of a Bioraptr dispenser.
2.12 -The assay plates were incubated at room temperature for 30min.
2.13 -Read the luminescence using ViewLux plate reader. (Exposure time: 20sec)