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 (Endogenous)	vMCF-7 (Misidentified as BG1)	Human	Breast carcinoma	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
-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 Aldrich / 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	-Corning	-Corning / 7464
-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
-CellTiter-Fluor(TM) Cell Viability Assay	-Promega	-Promega / G6082

-Recovery Cell culture Freezing Medium	-Invitrogen	-Invitrogen / 12648
-Beta-Estradiol (Agonist control compound)	-Sigma Aldrich	-Sigma Aldrich / E8875
-ICI 182,780 (Antagonist control compound)	-Sigma Aldrich	-Sigma Aldrich / I4409
-Tetraoctyl ammonium bromide (Viability positive control compound)	-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/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 in 4uL/well into 1536-well tissue treated white/solid bottom plates using an 8 tip dispenser (Multidrop).

2.3 -Incubate the assay plates for 22hrs at 37C and 5% CO2.

2.4 -First 1uL of 10.0nM (final concentration) ICI-182,780 (ER-Antagonist) or assay buffer was added using two separate tips of a dispenser (BioRAPTR).

2.5 –Then transfer 23nL of compounds from the library collection and positive control to the assay plates by using a Pintool station.

2.6 -Incubate the assay plates for 22hrs at 37C and 5% CO2.

2.7 -After 21hrs of incubation, 1ul of CellTiter-Fluor(TM) Cell Viability Assay reagent was added using a single tip of a dispenser (BioRAPTR).

2.8 -Incubate the assay plates at 37C and 5% CO2 for 1hr.

2.9 -Measure fluorescence signal by ViewLux plate reader (Exposure time = 1sec).

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

2.11 -Incubate the plates at room temperature for 30min.

2.12 -Measure luminescence signal by ViewLux plate reader (Exposure time = 30sec).

3. Assay Performance

ERα-BG1 ICI-182,780 added	Online Validation Agonist (Mean ± SD)	Online Validation Viability (Mean ± SD)
EC50 ICI-182,780-free β-estradiol	0.019nM ±0.005 (n = 27)	NA
EC50 β-estradiol with 10.0nM ICI-182,780	0.94nM ±0.11 (n = 27)	NA
S/B	8.79 ± 1.58	7.07 ± 0.20
CV (%)	15.72 * ± 3.53 (n = 20)	6.39 * ± 0.51 (n = 21)
Z’	0.82 ± 0.03	0.85 ± 0.03

* CV values shown represent average of all assay plates excluding the top two concentration plates