

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

DOCUMENT: ER-alpha-BG1_TOX21_SLP_Version1.0
TITLE: Protocol of ER-alpha-BG1 BG1 Cell-based Assay for High-throughput Screening

ASSAY REFERENCES:

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:

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:

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

ERα-BG1 (4-Hydroxy Tamoxifen; Antagonist control)	Online Validation Antagonist (Mean \pm SD)
IC ₅₀	0.04 \pm 0.004 μ M (n = 27)
S/B	7.88 \pm 0.39
CV (%)*	8.27 \pm 5.78 (n = 18)
Z'	0.73 \pm 0.10

DMSO plates and low concentration plates only.

*CV values shown represent average of