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)	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
Beta-Estradiol (Agonist control compound)	Sigma	Sigma/E8875

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 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 -Incubate the plates for 22hrs at 37C and 5% CO2
2.6 -Add 5ul of ONE-Glo reagent
2.7 -Incubate the plates at room temperature for 30min
2.8 -Measure luminescence by ViewLux plate reader

3. Assay Performance

ERα-BG1 (Beta-Estradiol; Agonist control)	**Online Validation Agonist (Mean ± SD)**
EC50	0.17 ± 0.12 nM (n = 27)
S/B	2.58 ± 0.17
CV (%)*	14.79 ± 4.65 (n = 18)
Z'	0.36 ± 0.16

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