Protocol of GR-BLA HeLa Cell-based Assay for High-throughput Screening

DOCUMENT: GR-BLA_TOX21_SLP_Version1.0

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ASSAY RFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
Glucocorticoid receptor (Endogenous)	HeLa	Human	Cervical carcinoma	Beta-lactamase reporter	Invitrogen	NR signaling

QUALITY CONTROL PRECAUTIONS:

1. -cells should not be grown more than 80-85% confluence
2. -The cell performance is affected is they are more confluent
3. -handle 1536 well plate black clear bottom plates carefully by sides

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
-DMEM with Glutamax	-Invitrogen	-10566
-Dialyzed FBS	-Invitrogen	-26400
-NEAA	-Invitrogen	-11140
-HEPES	-Invitrogen	-15630
-Sodium Pyruvate	-Invitrogen	-11360
-Pennicillin/Streptomycin	-Invitrogen	-15140
-Blasticidin	-Invitrogen	-R21001
-Mutidrop	-Thermofisher	-
-BiorapTR dispenser	-Beckman Coulter	-
-Envision plate reader	-Perkin Elmer	-
-LiveBLAzer B/G FRET substrate	-Invitrogen	-K1030
-Opti MEM	-Invitrogen	-11058

PROCEDURE:

1. Cell handling:
   1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
-Recovery Cell freezing medium	-	-	-	-100%
-DMEM with Glutamax	-90%	-	-90%	-
-Dialyzed FBS	-10%	-1%	-10%	-
-NEAA	-0.1 mM	-0.1 mM	-0.1 mM	-
-HEPES	-25 mM	-25 mM	-25 mM	-
-Na-Pyruvate	-1 mM	-1 mM	-1 mM	-
-Penn/Strep	-1 %	-1 %	-1 %	-
-Blasticidin	-5 ug/ml	-	-	-
-OptiMEM	-	-99%	-	-

   1.2. Thawing method

      1.2.1 -Place 14 mL of pre-warmed thaw medium into a 15 ml conical tube
      1.2.2 -Remove the vial of cells to be thawed from liquid nitrogen and thaw rapidly by placing at 37°C in a water bath with gentle agitation for 1-2 minutes. Do not submerge vial in water.
      1.2.3 -Mix the entire content of the vial to 14 ml of pre-warmed medium and centrifuge to remove DMSO
      1.2.4 -Discard the supernatant and transfer the precipitated cells to T175 flask using 30 ml thawing medium

   1.3. Propagation method

      1.3.1 -Detach the cells from the flask using TrypLExpress
      1.3.2 -The cells are re-seeded in T-175 flask at 1-1.3 million

2. Assay Protocol

   2.1 -Spin down the cells after rinsing the cells with DPBS and trypsinizing
   2.2 -Resuspend the pellet with assay medium
   2.3 -Plate the cells in black-clear bottom 1536 well plate at 1500/well/6uL through 8 tip Multidrop plate dispenser
   2.4 -Incubate for 4 hrs at 37ºC / 99% Humidity / 5% CO2
   2.5 -Transfer 23nL of compounds from the library collection and positive control to the assay plates through pintool
   2.6 -Add 1 uL of buffer and 1uL of Agonist concentration to respective columns as per plate map
   2.7 -Incubate for 18 hrs at 37ºC / 99% Humidity / 5% CO2
   2.8 -Add 1uL of CCF4 (FRET Substarte) dye using a single tip plate dispenser (Bioraptr)
   2.9 -Incubate at room temperature for 2 hrs in dark

2.10 -Read the fluorescence intensity through Envision plate reader using Beta-Lactamaze protocol optimized for this cell type
2.11 -Add 3 uL of Cell Titer Glo and Incubate at room temperature for 0.5 hrs in dark
2.12 -Read on ViewLux protocol optimized for this cell type

3. Assay Performance

GR-bla Antagonist (Mifepristone)	Online Validation (Mean ± SD)	Online Validation Viability (Mean ± SD)
IC50	3.85 ± 1.40 nM (n = 27)	N/A
S/B	1.89 ± 0.12	91.95 ± 4.48
CV (%)	4.34 ± 0.42* (n = 21)	7.35 ± 1.11 (n = 21)
Z'	0.54 ± 0.12	0.80 ± 0.04

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