

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

**DOCUMENT:** GR-BLA\_TOX21\_SLP\_Version1.0

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**ASSAY REFERENCES:**

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
-multidrop	-Thermofisher	-
-BiorapTR dispenser	-Beckman Coulter	-Beckman Coulter
-Envision plate reader	-Perkin Elmer	-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 TrypLEExpress

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 37°C / 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 -Incubate for 18 hrs at 37°C / 99% Humidity / 5% CO2

2.7 -Add 1uL of CCF4 (FRET Substrate) dye using a single tip plate dispenser (Bioraptr)

2.8 -Incubate at room temperature for 2 hrs in dark

2.9 -Read the fluorescence intensity through Envision plate reader using Beta-Lactamase protocol optimized for this cell type

### 3. Assay Performance

GR-bla Agonist (Dexamethasone)	Online Validation (Mean $\pm$ SD)
EC50	3.60 $\pm$ 0.78 nM (n = 27)
S/B	2.95 $\pm$ 0.70
CV (%)	3.54 $\pm$ 0.40* (n = 21)
Z'	0.64 $\pm$ 0.06

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