

# Protocol of PPAR-delta-BLA HEK 293T Cell-based Assay for High-throughput Screening

**DOCUMENT:** PPAR-delta-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
PPARdelta: LBD (Recombinant)	HEK 293T	Human	Embryonic kidney cells	Beta-lactamase reporter	Invitrogen	NR signaling

## QUALITY CONTROL PRECAUTIONS:

1. -The cells should not be grown more than 80-85% confluence
2. -The cell performance is affected is they are more confluent
3. - Do not leave cells in Trypsin for more than 5 min at RT
4. -handle 1536 well plate black clear bottom plates carefully by sides

## MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
-DMEM+Glutamax	-Invitrogen	-10569
-Phenol Red free DMEM	-Invitrogen	-21063
-Dialyzed FBS	-Invitrogen	-26400
-Charcoal Stripped FBS	-Invitrogen	-12676
-Sodium Pyruvate	-Invitrogen	-11360
-Pen-Strep	-Invitrogen	-15140
-NEAA	-Invitrogen	-11140
-HEPES	-Invitrogen	-15630
-HygromycinB	-Invitrogen	-10687
-Zeocin	-Invitrogen	-R25001
-Multidrop	-Thermofisher	-
-BiorapTR	-Beckman Coulter	-

-Envision Plate Reader	-Perkin Elmer	-
-LiveBLAzer B/G FRET substrate	-Invitrogen	-K1030

## PROCEDURE:

### 1. Cell handling:

#### 1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
-Recovery Cell Freezing Medium	-	-	-	-100%
-DMEM+Glutamax	-90%	-	-90%	undefined
-Phenol Red free DMEM	-	-98%	-	-
-Dialyzed FBS	-10%	-	-10%	undefined
-Charcoal-Stripped FBS	-	-2%	-	-
-Penn-strep	-1%	-1%	-1%	-
-Sodium Pyruvate	-	-	-1 mM	-
-HEPES	-25 mM	-	-	-
-NEAA	-0.1 mM	-	-0.1 mM	-
-Hygromycin	- 80 ug/ml	-	-	-
-Zeocin	-100 ug/ml	-	-	-

#### 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 2.5-4 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 followed by filtering through cell strainer and adjust the required cell density

- 2.3 -Plate the cells in black-clear bottom 1536 well plate at 3000/well/6uL for agonist mode and 3000cells/well/5 ul for antagonist mode through 8 tip Multidrop plate dispenser
- 2.4 -Incubate for 5 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 -Add 1 uL of buffer and 1uL of Agonist concentration to respective columns as per plate map for antagonist mode
- 2.7 -Incubate for 17 hrs at 37°C / 99% Humidity / 5% CO2
- 2.8 -Add 1uL of CCF4 (FRET Substrate) 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-Lactamase 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 for both agonist and antagonist mode
- 2.12 -Read on ViewLux protocol optimized for this cell type for both agonist and antagonist mode

### 3. Assay Performance

PPAR $\delta$ -bla Agonist (L-165,041)	Online Validation (Mean $\pm$ SD)	Online Validation Viability (Mean $\pm$ SD)
EC50	0.03 $\pm$ 0.006 $\mu$ M (n = 27)	N/A
S/B	4.90 $\pm$ 0.14	154.63 $\pm$ 29.60
CV (%)	8.50 $\pm$ 0.64* (n = 20)	7.20 $\pm$ 0.34* (n = 20)
Z'	0.71 $\pm$ 0.03	0.85 $\pm$ 0.03

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