Protocol of Luciferase Biochemical Assay for High-throughput Screening

DOCUMENT: Luc-Biochem_TOX21_SLP_Version1.0

TITLE: Protocol of AP1-BLA ME-180 Cell-based Assay for High-throughput Screening

ASSAY RFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
Luciferase	N/A	N/A	N/A			

QUALITY CONTROL PRECAUTIONS:

1. -The cells should not be grown more than 80-85% confluence
2. -The cell performance is affected if 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	-11965
-Dialyzed FBS	-Invitrogen	-26400
-NEAA	-Invitrogen	-11140
-HEPES	-Invitrogen	-15630
-Sodium Pyruvate	-Invitrogen	-11360
-Opti-MEM	-Invitrogen	-11058
-Penicillin Streptomycin	-Invitrogen	-15140
-0.25 Trypsin-EDTA	-Invitrogen	-25300
-Multidrop	-Thermofisher	-
-BiorapTR	-Beckman Coulter	-
-Envision	-Perkin Elmer	-
-LiveBLAzer B/G FRET substrate	-Invitrogen	-K1030
-Blasticidin	-Invitrogen	-R21001

PROCEDURE:

1. Cell handling:
   1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
--Recovery Cell Freezing Medium	-	-	-	-100%
-OptiMEM	-	-99.5%	-	-
-Dialyzed FBS	-10%	-0.5%	-10%	-
-NEAA	-0.1 mM	-0.1 mM	-0.1 mM	-
-HEPES	-25 mM	-	-25 mM	-
-Sodium Pyruvate	-	-1 mM	-	-
-Penicillin- Streptomycin	-1%	-1%	-1%	-
-Blasticidin	-5 ug/ml	-	-	-
-DMEM+ Glutamax	-90%	-	-90%	-

   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 supernantent 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 0.5 % Trypsin
      1.3.2 -The cells are re-seeded in T-175 flask at 3-4 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 2000/well/6uL for agonist mode through 8 tip Multidrop plate dispenser
   2.4 -Incubate for Overnight 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 -Incubate for 5 hrs at 37ºC / 99% Humidity / 5% CO2
   2.7 -Add 1uL of CCF4 (FRET Substarte) 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-Lactamaze protocol optimized for this cell type
2.10 -Add 3 uL of Cell Titer Glo and Incubate at room temperarture for 0.5 hrs in dark
2.11 -Read on ViewLux protocol optimized for this cell type

3. Assay Performance

Luc-Biochem (PTC 124)	**Online 10K Screen (Mean ± SD)**
IC50	7.1 ng/ml ± 0.0020 (n =408)
S/B	63.06 ± 7.31 (n =48)
CV (%)	4.45 ±3.30 * (n =48)
Z'	0.92 ± 0.03 (n =48)

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