Protocol of HRE-bla ME-180 Cell-based Assay for High-throughput Screening

DOCUMENT: HRE-bla_TOX21_SLP_Version1.0

TITLE: Protocol of HRE-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
Hypoxia/HIF-1 alpha	ME-180	Human	Cervix	Beta-lactamase reporter	Invitrogen	Stress response

QUALITY CONTROL PRECAUTIONS:

1. -Cells were passaged twice before running an assay (once for thawing and once splitting into culture medium)
2. -The assay should be performed in black-clear bottom 1536 well plates, so the bottom of the plates should not be touched

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
-DMEM	-Invitrogen	-Invitrogen/11965
-Opti-MEM	-Invitrogen	-Invitrogen/11058
-Dialyzed FBS	-Invitrogen	-Invitrogen/26400
-NEAA	-Invitrogen	-Invitrogen/11140
-Sodium pyruvate	-Invitrogen	-Invitrogen/11360
-HEPES	-Invitrogen	-Invitrogen/15630
-Penn-strep	-Invitrogen	-Invitrogen/15140
-Blasticidin S HCl	-Invitrogen	-Invitrogen/A11139-03
-Recovery Cell Culture Freezing Medium	-Invitrogen	-Invitrogen/12648
-0.25% Trypsin-EDTA	-Invitrogen	-Invitrogen/25200
-LiveBLAzer B/G FRET substrate (CCF4-AM)	-Invitrogen	-Invitrogen/K1028
-Solution D	-Invitrogen	-Invitrogen/K1157
-CellTiter-Glo Luminescent Cell Viability Assay	-Promega	-Promega/G8462
-Black-clear bottom 1536 well plates	-Greiner	-Greiner/789092F
-BioRAPTR FRD dispenser	-Beckman Coulter	-Beckman Coulter
-Multidrop COMBI	-Thermo Electron	-Thermo Electron

	Corporation	Corporation
-Envision Plate Reader	-Perkin Elmer	-Perkin Elmer
-ViewLux Plate Reader	-Perkin Elmer	-Perkin Elmer

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
-DMEM	-90%	-	-90%	-
-Opti-MEM	-	-99.5%	-	-
-Dialyzed FBS	-10%	-0.5%	-10%	-
-NEAA	-0.1mM	-0.1mM	-0.1mM	-
-Sodium pyruvate	-1mM	-1mM	-1mM	-
-HEPES	-25mM	-10mM	-25mM	-
-Penn-strep	-100U/ml-100 ug/ml	-100U/ml-100 ug/ml	-100U/ml-100 ug/ml	-
-Blasticidin S HCl	-5 ug/mL	-	-	-
-Recovery Cell Culture Freezing Medium	-	-	-	-100%

1.2. Thawing method

1.2.1 -1ml frozen cells of HRE-bla ME180 were taken in pre-warmed 9ml of thaw medium for centrifuging.
1.2.2 -2-3ml of the thaw medium is taken to resuspend the pellet
1.2.3 -The cells were seeded in T-225 flask at 5 million

1.3. Propagation method

1.3.1 -Rinse the cells with DPBS and detach them by using 0.25% Trypsin and centrifuge
1.3.2 -The cells are further passaged at a density of 25 million cells per 5-layer flask

2. Assay Protocol

2.1 -Plate the cells in black-clear bottom 1536 well plate at 2000/well/6uL of assay medium through 8 tip of a plate dispenser (Multi drop)
2.2 -Incubate at 37C for 5 hrs
2.3 -Transfer 23nL of compounds from the library collection and positive control through pintool
2.4 -Incubate at 37C for 17 hrs
2.5 -Add 1uL of CCF4 dye (Solution A + B+ C +D at 6uL + 60uL + 924uL +10uL respectively) using a single tip of a plate dispenser (Bioraptr)
2.6 -Incubate at room temperature for 2hrs
2.7 -Read the fluorescence intensity through Envision plate reader
2.8 -Add 4uL of CellTiter-Glo reagent using a single tip of a plate dispenser (Bioraptr)
2.9 -Incubate at room temperature for 30 min
2.10 -Read the luminescence through ViewLux plate reader

3. Assay Performance

HRE-bla	Online Validation Agonist (Mean ± SD)	Online Validation Viability (Mean ± SD
EC50	61.91 ± 16.3 uM (n = 24)	NA
S/B	4.51 ± 0.13	23.98 ± 0.32
CV (%)*	3.67 ± 0.29	5.94 ± 1.29
Z'	0.74 ± 0.05	0.84 ± 0.03

* CV values shown represent average of all assay plates excluding the top concentration plates.