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

DOCUMENT: HSE-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
Hsp70	HeLa	Human	Cervical carcinoma	FRET	Invitrogen	Stress response

QUALITY CONTROL PRECAUTIONS:

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
-Fetal bovine serum, dialyzed	-Invitrogen	-Invitrogen, 26400
-Nonessential amino acids (NEAA)	-Invitrogen	-Invitrogen, 11140
-Penicillin/Streptomycin (antibiotic)	-Invitrogen	-Invitrogen, 15140
-Sodium Pyruvate	-Invitrogen	-Invitrogen, 11360
-CCF4 dye	-Invitrogen	-Invitrogen, K1030
-DMEM	-Invitrogen	-Invitrogen, 10564
-Recovery Cell Freezing Media	-Invitrogen	-Invitrogen, 12648
-Blasticidin	-Invitrogen	-Invitrogen, A11139-03
-OptiMEM	-Invitrogen	-Invitrogen, 11058

PROCEDURE:

1. Cell handling:
   1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
-DMEM	-90%	-	-90%	-

-Dialyzed FBS	-10%	-0.5%	-10%	-
-1 mM Sodium Pyruvate	-1%	-1%	-1%	-
-0.1 mM NEAA	-1%	-1%	-1%	-
-100 U/ml Penn-Strep	-1%	-1%	-1%	-
-Blasticidin	-500 ul	-	-	-
-OptiMEM	-	-99.5	-	-
-Recovery Cell Freezing Media	-	-	-	-100%

1.2. Thawing method

1.3. Propagation method

2. Assay Protocol

2.1 -1. Plate the cells at 1.5k/6uL/well in black-clear bottom 1536 well plates with assay medium.
2.2 -2. Incubate for an overnight at 37ÂºC / 99% Humidity / 5% CO2.
2.3 -3. Pin the compounds.
2.4 -4. Incubate at 37ÂºC / 99% Humidity / 5% CO2 for 5- 6hrs.
2.5 -5. Add 1uL of CCF4 dye (Solution A+B+C+D) and incubate for 2-2.5 hrs at RT and read the fluorescence through Envision. Ex 405 nm Em 460 and 530 nm.
2.6 -6. While running the plate on Envision, please run optimization for gain in advanced mode using one well with max signal for quick optimization.

3. Assay Performance

HSP-bla Agonist (17AAG)	**Online Validation (Mean ± SD)**
EC50	0.08 ± 0.01 µM (n = 27)
S/B	2.94 ± 0.17
CV (%)	4.02 ±0.40 * (n = 20)
Z’	0.78 ± 0.01

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