Protocol of P53-BLA-HLM HCT-116 Cell-based Assay for High-throughput Screening

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ASSAY RFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
P53	HCT-116	Human	Colon carcinoma	Beta-lactamase reporter	Invitrogen	Stress response

QUALITY CONTROL PRECAUTIONS:

1. Handle the 1536-well, black-wall, clear-bottom assay plate by the sides; do not touch the clear bottom of the assay plate.

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
McCoy's 5A Medium	Invitrogen	Invitrogen, 16600-108
Opti-MEN Reduced Serum Medium	Invitrogen	Invitrogen, 11058-021
Fetal bovine serum, dialyzed	Invitrogen	Invitrogen, 26400
Nonessential amino acids (NEAA)	Invitrogen	Invitrogen, 11140
Penicillin/Streptomycin (antibiotic)	Invitrogen	Invitrogen, 15140
DPBS	Invitrogen	Invitrogen, 14190
Sodium pyruvate	Invitrogen	Invitrogen, 11360
0.25% Trypsin/EDTA	Invitrogen	Invitrogen, 25300
Blasticidin (antibiotic)	Invitrogen	Invitrogen, R210
DMSO	AMRESCO	KD Medical, RGE-3070
Recovery Cell Culture	Invitrogen	Invitrogen, 12648
LiveBLAzer FRET B/G Loading Kit:	Invitrogen	Invitrogen , K1030
Solution D	Invitrogen	Invitrogen, K1157
Aflatoxin B_1	sigma	A6636
Human liver microsomes	XENOTECH	H0610

Black-wall, clear-bottom, 1536-well assay plates	Greiner Bio-One	Greiner, 789092-F
PinTool	Kalypsys	-
BioRAPTR, Microfluidic Workstation	Beckmen	-
EnVision plate reader	Perkin Elmer	-
Centrifuge	Sorvall legend XTR	Thermo Fisher Science, 75004520

PROCEDURE:

1. Cell handling:
1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
McCoy's 5A medium	90%	-	90%	-
Opti-MEN	-	99.5%	-	-
Dialyzed FBS	10%	0.5%	10%	-
NEAA	-	0.1 mM	-	-
Sodium pyruvate	-	1mM	-	-
Penicillin-Streptomycin	100 U/mL-100 µg/mL	100 U/mL-100 µg/mL	100 U/mL-100 µg/mL	-
Blasticidin (antibiotic)	5 µg/mL	-	-	-
Recovery Cell Culture	-	-	-	100%

1.2. Thawing method

1.2.1 Place 14 mL of pre-warmed thaw medium into a 15 mL of conical tub.
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.
1.2.3 Decontaminate the vial by wiping with 70% ethanol before opening in a biological safety cabinet.
1.2.4 Transfer the vial contents drop-wise into 14 mL of thaw medium in a sterile 15-mL conical tub.
1.2.5 Centrifuge cells at 900 rpm for 4 minutes and resuspend in thaw medium.
1.2.6 Transfer contents to the T75 tissue culture flask containing Thaw Medium and place flask in a humidified 37°C/5% CO_2 incubator.
1.2.7 Switch to growth medium at first passage.

1.3. Propagation method

1.3.1 Aspirate medium, rinse once in DPBS, add 0.25% Trypsin/EDTA (3 mL for a T75 flask and 5 mL for a T175 flask and 7.5 mL for T225 flask) and swirl to coat the cell evenly.
1.3.2 Add an equal volume of Growth Medium to inactivate Trypsin after 2-3 minutes incubation at 37°C.
1.3.3 Centrifuge cells at 900 rpm for 4 minutes and resuspend in growth medium.

1.3.4 Cell should be passage or fed at least twice a week.

2. Assay Protocol

2.1 Harvest cells from culture in growth medium and resuspend in assay medium.
2.2 Dispense 4000 cells/3μL/well into 1536-well black/clear bottom plates using a Multidrop dispenser.
2.3 After the cells were incubated at 37°C and 5% CO2 for 5 hours, 23 nL of compounds dissolved in DMSO, positive controls or DMSO were transferred to the assay plate by a PinTool resulting in a 217-fold dilution. Following compound addition, 3 μL of human liver microsomes at final concentration of 0.5 mg/mL and 1μL of NADPH at 0.5 mg/mL were transferred to the assay plate.
2.4 Incubate the plates for 16 hours at 37°C, 5% CO2.
2.5 Add 1 μL of 8X LiveBLAzer FRET B/G (CCF4-AM) Substrate Mixture to each well using a BioRAPTR dispenser and incubate the plates at room temperature for 2 hours.
2.6 Measure fluorescence intensity at 460 and 530 nm emission and 405 nm excitation by an Envision detector. Data is expressed as the ratio of 460nm/530nm emissions.
2.7 Add 3 uL of Cell-titer Glo to each well using BioRAPTR and incubate the plates at room temperature for 30 min
2.8 Measure luminescence intensity by a VewLux plate reader.

3. Assay performance: