Protocol of FXR-BLA HEK 293T 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
Farnesoid X receptor: LBD (Recombinant)	HEK 293T	Human	Embryonic kidney	Beta-lactamase reporter	Invitrogen	NR signaling

QUALITY CONTROL PRECAUTIONS:

1. Cells should be grown to reach 80 to 90% confluence

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
DMEM, with GlutaMAX	Invitrogen	10569
DMEM, phenol red-free	Invitrogen	21063
Fetal bovine serum, dialyzed	Invitrogen	26400
Nonessential amino acids (NEAA)	Invitrogen	11140
Penicillin/Streptomycin (antibiotic)	Invitrogen	15140
DPBS	Invitrogen	14190
Sodium pyruvate	Invitrogen	11360
HEPES (1 M, pH 7.3)	Invitrogen	15630
0.05% Trypsin/EDTA	Invitrogen	25300
Hygromycin (antibiotic)	Invitrogen	10687
Zeocin (antibiotic)	Invitrogen	R25001
LiveBLAzer FRET B/G Loading Kit: Solution A, B and C	Invitrogen	K1030
Recovery Cell Culture Freezing Medium	Invitrogen	12648
Fetal bovine serum (FBS), charcoal stripped	Invitrogen	12676

Chenodeoxycholic acid (CDCA)	Sigma	C9377
Black, clear-bottom, 1536-well assay plates	Greiner BioOne	789092-F
PinTool	Kalypsys	-
BioRAPTR, Microfluidic Workstation	Beckmen	-
EnVision plate reader	Perkin Elmer	-

PROCEDURE:

1. Cell handling:
   1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
DMEM with GlutaMAX	90%	-	90%	-
DMEM, phenol red-free	-	98%	-	-
Dialyzed FBS	10%	-	10%	
Charcoal stripped FBS	-	2%	-	-
Sodium pyruvate	-	1mM	-	-
NEAA	0.1 mM	0.1mM	0.1 mM	-
HEPES (pH 7.3)	25 mM	-	25 mM	-
Penicillin/Streptomycin	100U/mL/100µg/mL	100U/mL/100µg/mL	100U/mL/100µg/mL	-
Hygromycin (antibiotic)	100 µg/mL	-	-	-
Zeocin (antibiotic)	100 µg/mL	-	-	-
Recovery Cell Culture Freezing Medium	-	-	--	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 0.05% Trypsin/EDTA 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 at least twice a week.

2. Assay Protocol

2.1 Harvest cells from culture and resuspend in assay medium.
2.2 Dispense 5000 cells/5µL/well into 1536-well tissue treated black, clear-bottom plates using a Multi-drop dispenser.
2.3 After the cells were incubated at 37°C for 5 hours, 23 nL of control or compounds dissolved in DMSO were transferred to the assay plate by a PinTool resulting in a 217-fold dilution.
2.4 Incubate the plates for 16 hours at 37°C.
2.5 Add 1 µL of 6X LiveBLAzer-FRET B/G (CCF4-AM) Substrate Mixture to each well using a BioRAPTR dispenser.
2.6 After two hours incubation at room temperature, 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.

3. Assay Performance

FXR-bla-Agonist (CDCA)	Online Validation (Mean ± SD)
EC50	19.84 ± 2.95 µM (n = 27)
S/B	3.97 ± 0.47 (n = 27)
CV (%)	3.62 ± 0.27* (n=18)
Z'	0.72 ± 0.09 (n = 27)

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