Protocol of Caspase-Glo 3/7 HepG2 Cell-based Assay for High-throughput Screening

DOCUMENT: Caspase-Glo 3/7 HepG2_TOX21_SLP_Version1.0

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ASSAY RFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
Caspase-3/7 activity	HepG2	Human	Hepatocellular carcinoma	Luminescence	Caspase-Glo 3/7 assay from Promega Corporation	Apoptosis

QUALITY CONTROL PRECAUTIONS:

1. -Maintain cells below 85-90% confluence.

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
-EMEM Medium	-ATCC	-ATCC/30-2003
-Fetal Bovine Serum	- Hyclone	- Hyclone/SH30071.03
-Penicillin & Streptomycin	-Invitrogen	-Invitrogen/15140
-Recovery Cell culture Freezing Medium	-Invitrogen	-Invitrogen/12648
-0.05% Trypsin-EDTA	-Invitrogen	-Invitrogen/25300
-Staurosporine (Agonist control compound)	-Sigma	-Sigma/S4400
-Tetraoctyl ammonium bromide (Viability control compound)	-Sigma	-Sigma/294136
-1536-well white solid plates	-Corning	-Corning/7464
-MULTIDROP COMBI	-Thermo Electron Corporation	-Thermo Electron Corporation
-BioRAPTR FRD	-Beckman Coulter	-Beckman Coulter
-ViewLux Plate Reader	-Perkin Elmer	-Perkin Elmer
-CellTiter-Fluor(TM) Assay System	-Promega	-Promega/G6082

-ONE-Glo(TM) Luciferase Assay System	-Promega	-Promega/E6130

PROCEDURE:

1. Cell handling:
1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
-EMEM Medium	-90%		-90%	
-Fetal Bovine Serum	-10%		-10%	
-Penicillin & Streptomycin	-100U/ml & 100ug/ml	-100U/ml & 100ug/ml	-100U/ml & 100ug/ml	
-Recovery Cell culture Freezing Medium				-100%

1.2. Thawing method

1.2.1 -Thaw a vial of cells in 9ml of pre-warmed thaw medium and then centrifuge
1.2.2 –Re-suspend the pellet with the thaw medium and seed at 2 million cells per T-75 flask

1.3. Propagation method

1.3.1 -Trypsinize cells from the culturing flask and centrifuge and then re-suspend cells in culture medium
1.3.2 -Passage cells at 2-3 million per T-225 flask

2. Assay Protocol

2.1 -Trypsinize cells from the culturing flask and centrifuge and then re-suspend cells in culture medium at a density of 0.4 X 10^6 cells/mL
2.2 -Dispense 2000 cells/5uL/well into 1536-well tissue treated white/solid bottom plates using a 8 tip dispenser (Multidrop)
2.3 -Incubate the plates for 5hr at 37C and 5% CO2
2.4 -Transfer 23nL of compounds from the library collection (0.59nM to 92uM) and positive control through Pintool
2.5 -Incubate the plates for 17hr at 37C and 5% CO2
2.6 –After 16hrs of incubation at 37C, add 1ul of CellTiter-Fluor using single tip dispense (Bioraptr)
2.7 -Incubate the plates for 1hr at 37C and 5% CO2
2.8 –Read fluorescence (exposure time = 3sec) intensity using ViewLux plate reader
2.9 -Then add 4ul of Caspase-Glo 3/7 (R) assay reagent using a single tip dispense (Bioraptr)
2.10 -Incubate the plates at room temperature for 30min
2.11 -Read luminescence (exposure time = 1sec) intensity using ViewLux plate reader

3. Assay Performance

Online Validation	**HepG2 cells** Caspase-Glo® 3/7 assay	**HepG2 cells** Viability assay
CV*	6.91 ± 0.42 (n = 23)	4.61 ± 0.38 (n = 24)
S/B	15.41 ± 0.68	4.86 ± 0.20
Z	0.85 ± 0.03	0.83 ± 0.01

♂*CV values shown represent average of all plates excluding high compound concentration plates.