

Protocol of Real Time Viability Assay Using HepG2 Cell Line

DOCUMENT: RT Viability_TOX21_SLP_Version1.0

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ASSAY REFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
Cell viability/Cytotoxicity	HepG2	Human	Hepatocellular carcinoma	Luminescence /Fluorescence	Promega	Cytotoxicity

QUALITY CONTROL PRECAUTIONS:

Cells should be grown to reach 60 to 90% confluence.

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vender/Catalog Number
EMEM	ATCC	30-2003
Fetal bovine serum	Hyclone	SH30071-03
Penicillin/Streptomycin	Invitrogen	15140
DPBS	Invitrogen	14190
0.25% Trypsin/EDTA	Invitrogen	
Recovery Cell Culture	Invitrogen	12648
CellTox Green Express Cytotoxicity Assay	Promega	G8731
RealTime-Glo MT Cell Viability Assay	Promega	G9713
Blackwall, 1536 well assay plates	Greiner BioOne	789078

PROCEDURE:

1. Cell handling:

1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium
EMEM	89%	89%	89%
Fetal bovine serum	10%	10%	10%
Penicillin/Streptomycin	1%	1%	1%

1.2. Thawing method

1.2.1 Place 14 mL of pre-warmed thaw medium into a T75 flask

1.2.2 Remove the vial of cells from liquid nitrogen and thaw rapidly by placing at 37°C in a water bath with gentle agitation for 1-2 minutes. Do not submerge vial in water.

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 dropwise into 10 mL of Thaw Medium in a sterile 15mL conical tub
- 1.2.5 Centrifuge cells at 1000 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% CO₂ incubator.

1.3. Propagation method

- 1.3.1 Aspirate medium, rinse once in DPBS, add 0.25% 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 1000 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 HepG2 cells and adjust the cell density to 600cells/well/6uL.
- 2.2 To prepare mixture of cell suspension and reagents for one 1536well plate, add 10ul of 1000x CellTox Green dye, 10ul of 1000x NanoLuc, and 10ul of 1000x Metabolic Cell Viability Substrate per 10ml of cell suspension. This dilution brings each component to 1x concentration.
- 2.3 Plate 6ul of mixture of reagents and cell suspension prepared in step 2 into each wells of 1536-well tissue culture treated black solid bottom plate using a Multidrop Dispenser.
- 2.4 After the cells were incubated at 37°C 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.
- 2.5 Incubate the plates at 37C, 5% CO₂
- 2.6 Measure fluorescence or luminescence after pinning at 0h, 8h, 16h, 24h, 32h and 40h time points by ViewLux.

3. Assay Performance

1) RealTime-Glo™ MT Cell Viability Assay

Online screening (n=136)	Cmp Treatment Time	8h	16h	24h	32h	40h
	IC50 (μM)	7.90±1.62	7.53±1.44	7.79±1.54	8.05±1.68	8.18±1.91
	CV% (DMSO)	3.31±0.81	2.60±0.62	2.24±0.72	2.47±1.83	2.86±1.32
	Z'	0.87±0.02	0.90±0.02	0.91±0.02	0.90±0.05	0.89±0.04
	S/B	26.14±1.02	27.18±1.02	27.56±1.11	27.15±4.15	27.53±1.54

2) CellTox™ Green Cytotoxicity Assay

Online screening (n=136)	Cmp Treatment Time	8h	16h	24h	32h	40h
	EC50 (μM)	7.59±1.48	6.18±1.27	5.87±1.10	6.43±1.00	6.40±0.96
	CV% (DMSO)	8.26±2.24	8.79±3.57	9.58±5.50	9.67±6.79	9.83±7.08
	Z'	0.73±0.07	0.72±0.09	0.71±0.10	0.78±0.08	0.78±0.10
	S/B	3.69±0.38	3.61±0.54	4.42±0.79	5.50±1.25	6.08±0.94