Protocol of Mitochondria Toxicity HepG2 Cell-based Assay for High-throughput Screening

DOCUMENT: Mitochondria Toxicity_TOX21_SLP_Version1.0

TITLE: Protocol of Mitochondria Toxicity HepG2 Cell-based Assay for High-throughput Screening

ASSAY RFERENCES:

Assay Target	Cell Lines	Species	Tissue of Origin	Assay Readout	Assay Provider	Toxicity Pathway
Mitochondrial membrane potential	HepG2	Human	Hepatocellular carcinoma	Fluorescence	Codex Biosciences	Stress response

QUALITY CONTROL PRECAUTIONS:

1. -Use black clear bottom plates

MATERIALS and INSTRUMENTS:

Supplies/Medium/Reagent	Manufacturer	Vendor/Catalog Number
-Eagle's MEM	-ATCC	-30-2003
-M-MPI	-codex	-CB-80600-010
- FBS	- Hyclone	-SH30071.03
- Penn-strep	- Invitrogen	-15140
-EnVision Multilabel Reader	- PerkinElmer	-2104-0010
-Recovery Cell Culture Freezing Medium	-GIBCO	-12648
-FCCP (Antagonist control compound)	-Sigma	-Sigma/C2920

PROCEDURE:

1. Cell handling:
   1.1. Media Required:

Component	Growth Medium	Assay Medium	Thaw Medium	Freezing Medium
-Eagle's MEM (ATCC, 30-2003)	-89%	-10%	-1%	-

-FBS (Hyclone, SH30071.03)	-89%	-10%	-1%	-
-1% Penn-strep (Invitrogen, 15140)	-89%	-10%	-1%	-
-Recovery Cell Culture Freezing Medium	-	-	-	-100%

1.2. Thawing method

1.2.1 -Add 10 ml of pre-warmed medium in a 15 ml falcon tube
1.2.2 -Remove the vial of cells to be thawed from liquid nitrogen and thaw rapidly by placing at 37C in a water bath with gentle agitation for 1-2 minutes. Do not submerge vial in water.
1.2.3 --Add the cells into the 10 ml. Wash once the cryogenic tube with 1 ml of medium and add it to the falcon tube
1.2.4 --Centrifuge for 5 minutes at 900 rpm
1.2.5 -Resuspend the cells in warm medium
1.2.6 -Count the cells using a Hemocytometer
1.2.7 -Add cells to T225 containing 40 ml of medium. Dilute if necessary

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 -wait 5 minutes at 37 C. Check under the microscope to ensure that most of the cells are detached
1.3.3 -Add an equal volume of Growth Medium to inactivate Trypsin. Transfer to a falcon tube. Centrifuge for 5 minutes at 900 rpm. Resuspend the cells in warm medium
1.3.4 -Pass the cells through a 40 um Cell Strainer
1.3.5 -Count the cells using a Hemocytometer. Dilute to desired concentration
1.3.6 -Add cells to T225 containing 40 ml of medium

2. Assay Protocol

2.1 -Plate HepG2 cells at 2000 per well in 5ul of culture medium
2.2 –Incubate for an overnight
2.3 -Add library and control compounds at 23nl
2.4 -Incubate for 1hr
2.5 -Add 5ul m-MPI dye
2.6 -Incubate at 37C for 30min
2.7 -Read the assay on EnVision Multilabel Reader

3. Assay Performance

MMP (FCCP; Antagonist control)	**Online Validation Antagonist (Mean ± SD)**
IC50	0.27 ± 0.05 μM (n = 27)
S/B	9.40 ± 0.88

CV (%)	7.84 ± 0.82* (n = 18)
Z'	0.77 ± 0.04

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