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

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| **DOCUMENT:** |  | Mitochondria Toxicity\_TOX21\_SLP\_Version1.0 |
| **TITLE:** |  | Protocol of Mitochondria Toxicity HepG2 Cell-based Assay for High-throughput Screening |

**ASSAY RFERENCES:**

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| --- | --- | --- | --- | --- | --- | --- |
| 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:**

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| --- | --- | --- |
| 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

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| --- | --- |
| **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.