MTT Cell Proliferation and Cytotoxicity Assay Kit

Catalog No.: abx090676

Size: 500 tests

Storage: Store all components at 2-8°C in the dark.

Application: For quantification of Cell Proliferation and Cytotoxicity in cell samples.

Introduction

Abbexa's MTT Cell Proliferation and Cytotoxicity Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Cell Proliferation and Cytotoxicity. Dehydrogenases from mitochondria reduce MTT to a purple crystalline formazan. Once dissolved in DMSO, the compound that has an absorption maximum at 570 nm. The intensity of purple color is proportional to the dehydrogenase activity, which can then be calculated relative to untreated controls.

Kit components

1. MTT (5X): 5 ml
2. MTT Diluent Buffer: 2 × 12.5 ml

Materials Required But Not Provided

1. 96-well microplate, PCR plate, or equivalent
2. Microplate reader (570 nm)
3. Microplate centrifuge
4. DMSO
5. Double distilled water
6. Pipette and pipette tips
7. Vials/tubes
8. Incubator

Protocol

A. Preparation of reagents

Bring all reagents to room temperature prior to use.

- **MTT Working Solution:** Dilute MMT (5X) 5-fold with MTT Diluent Buffer (for example, add 100 µl of 5X MTT to 400 µl of MTT Diluent Buffer and mix fully). If the MMT appears as a solid, warm in a water bath at 20-25°C until the reagent is completely liquid. MMT should have a yellow color; do not use if it appears as a grey-green color.

B. Assay Procedure

1. Set Blank, Control and Sample wells on the 96-well microplate and label accordingly.
2. **Cytotoxicity assay:** Add 100 µl of cell suspension sample, approximately 5000 cells, to the Sample wells. Add 100 µl of untreated cell suspension, approximately 5000 cells, to the Control wells.
   Cell proliferation assay: Add 100 µl of cell suspension sample, approximately 2000 cells, to the Sample wells. Add 100 µl of untreated cell suspension, approximately 2000 cells, to the Control wells.
   Note: Reducing agents, such as some antioxidants, may interfere with this kit. It is recommended to remove reducing agents from samples before use in the assay.
3. Add 100 µl of culture media to the Blank wells.
4. Culture cells according to typical conditions for the cell type.
5. Add 50 µl of MTT Working Solution to all wells and incubate for 1-4 hours in the same conditions as cell culture.
6. **Suspension cells:** Centrifuge the plate to pellet cells at the bottom of the wells.
7. Remove the liquid phase from each well. Do not touch the sides or bottom of the wells.
8. Add 150 µl of DMSO to dissolve the solid phase and mix with a microplate shaker.
9. Ensure there are no bubbles present in any of the wells. Read and record the absorbance at 570 nm with a microplate reader.

C. Calculation of Results

$$\text{Cell Survival Rate (\%)} = \frac{\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}}}{\text{OD}_{\text{control}} - \text{OD}_{\text{blank}}} \times 100$$

$$\text{Inhibition of Proliferation Rate (\%)} = \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}}{\text{OD}_{\text{control}} - \text{OD}_{\text{blank}}} \times 100$$

where:

$\text{OD}_{\text{sample}}$	OD value of the sample
OD_{blank}	OD value of the blank
$\text{OD}_{\text{control}}$	OD value of the control