EasyAssay™ MTT Cell Viability Assay Kit

(5000 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-88100-5000

www.zellx.de

Application:

Detection of Cell Viability, Proliferation & cytotoxicity

!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

Table of Contents

Please read this insert completely prior to using the product.

Background

Thiazolyl blue tetrazolium bromide also called MTT is a cell-permeable and positively charged tetrazolium dye commonly used as a sensitive and reliable method to assess cell viability, proliferation, cytotoxicity, and apoptosis. Nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cellular oxidoreductase of living cells reduces the MTT to its insoluble, dark blue MTT-formazan which can be colorimetrically measured at a wavelength of 570 nm (500-600 nm).

The MTT assay is a sensitive and reliable colorimetric assay for measuring cell metabolic activity and has been widely used to assess cell viability. However, as the enzymatic reduction of 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) to MTT-formazan is catalyzed by mitochondrial succinate dehydrogenase, the MTT assay is dependent on mitochondrial respiration and indirectly serves to assess the cellular energy capacity of a cell.

Intended use

Determination of cell viability, cell proliferation and cytotoxicity.

Materials supplied in the Kit

Component	*Quantity*
Thiazolyl Blue Tetrazolium Bromide (MTT)	250 mg
PBS tablet	1
0.2 µM filter	1

Storage instruction

All reagents should be stored at room temperature, except MTT which must be stored at 4°C.

Materials required but not supplied

Distilled water (dH_2O)

Dimethyl Sulfoxide (DMSO), We recommend to use our DMSO, Catalog Number ZXB-09-255, or Sigma-Aldrich Catalog Number D8418

Precision pipettes and disposable pipette tips

Sterile clear 96 well plate

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

General remarks

- Equilibrate all kit components to room temperature (RT) before use.
- The instruction must be strictly followed.
- Pipette tips should not be used more than once to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Assay preparation

i. **1X PBS Solution:** Prepare 1X PBS solution by dissolving 1 provided PBS tablet in 100 mL dH_2O. PBS solution can be stored at room temperature.

ii. **MTT Stock Solution:** Prepare MTT reagent by dissolving 250 mg MTT in 50 mL 1X PBS solution, and Filter it via 0,2 µM filter. MTT stock solution can be stored at -20°C for up to 2 years.

Sample preparation

i. Two to three days before the experiment, cultivate cells (0.5 - 2 x 10^4 cells/well) into a 96-well plate containing 100 µL/well of cell culture medium. The appropriate time of incubation may vary depending on the cell types and the desired chemical or physical treatments and must be evaluated by the end user.

ii. Each plate must be included at least two wells as background control which contain 10 µL of the MTT reagent + 90 µL complete media (without phenol red and serum).

- We strongly recommend to determine the optimal cell number and incubation time for your specific cells before performing a large number of MTT assays.

- Serum or phenol red in the culture medium can generate background and may interfere with result and must be avoided.

Assay Procedure

1. **Optional**: treat the cells with compounds of interest dissolves in an appropriate solvent for desired time period. In the case of treatment include control wells for the solvent, the cells must be treated with the same solvent without compounds of interest.
2. Discard the supernatant (either by aspiration for adherent cells or centrifugation at 1000 g and 4°C for 5 minutes for suspension cells) and resuspend cells in 90 µL complete media (without phenol red and serum).
3. Add 10 µL of the MTT reagent to each well. Mix gently for one minute.
4. Incubate the cells for 3-5 hours (adherent cells & suspension cells) at 37°C in a CO_2 incubator.
5. Add 100 µL of DMSO into each well.
6. Cover the plate with the foil and shake the plate shake for 15 minutes at room temperature.
7. Read the absorbance signal at 570 nm (500-600 nm is acceptable).

Calculations

i. Average the duplicate optical density (OD) readings for each sample.

ii. Subtract the background absorbance from the signal absorbance to obtain normalized absorbance values.

$$\%\ \boldsymbol{Relative\ Cytotoxicity} = \mathbf{100} \times \frac{\boldsymbol{OD\ background\ Control - OD\ Sample}}{\boldsymbol{OD\ background\ Control}}$$