MTT Assay Reagent, Ready-to-Use

M0200

Storage

MTT Reagent is stable for at least 2 years at - 20°C.

Repeated thawing and freezing will cause an increase in the background, which will interfere with your assay.

MTT Reagent is stable for 3-4 days in the dark at 2-4°C.

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Introduction

The MTT Assay Reagent provides a simple method for determining live cell numbers using a standard colorimetric plate readers. Determination of live cell numbers is often used to assess the rate of cell proliferation and cytotoxicity caused by drugs and cytotoxic agents. Among all non-radioactive viability assays, MTT assay developed by Mossman is one of the most versatile and popular assays. MTT is a tetrazolium salt that is turned into a purple formazan product after reduction by mitochondrial enzymes that are only present in metabolically active live cells, not in dead cells. The amount of formazan product generated is proportional to the number of living cells in the sample. At the end of the assay, the cells containing the formazan product are solubilized and then photometrically quantified at 570 nm.

Required Equipment and Materials

- Microplate reader (470 nm filter)
- 96-well plate
- CO_2 incubator
- 10 µl and 100 - 200 µl multi-channel pipettes
- DMSO (Dimethyl Sulfoxide)

Precautions

The MTT Reagent is ready to use and stable at 2-4°C in the dark for 2-4 days after thaw, provided there is no contamination. Care should be taken not to contaminate the MTT Reagent with cell culture medium during pipetting. We recommend that the appropriate volume required for each experiment be removed and aseptically placed into a separate clean tube and the stock bottle returned to -20°C in the dark. If the MTT Reagent is blue-green, do not use.

Cell Counting Assay

1. Plate cells into 96-well tissue culture plates. In general, cells should be seeded at densities between 5,000 and 10,000 cells per well in order to reach optimal density within 48 to 72 hours.
2. Carry out desired cell treatments. The final volume of culture medium in each well should be 100 uL, and the medium may contain up to 10% FBS.
3. If sediment is present in the MTT solution, heat the solution to 37°C and mix gently until a clear solution is obtained.
4. Add 10 uL MTT Reagent to the 100 uL of medium in each well. Mix by tapping gently on the side of the tray or shake briefly on an orbital shaker.
5. Incubate at 37°C for 4 hours. At high cell densities (>100,000 cells per well) the incubation time can be shortened to 2 hours.
6. Add 200 uL DMSO directly into the medium in each well and pipette up and down several times to dissolve the formazan salt. The final volume in the well will be 300 uL (a standard 96-well cell culture plate has a maximum volume of 400 uL).
7. Measure the absorbance signal on a spectrophotometer at 570 nm. Measure background absorbance at 630 nm. Subtract background absorbance from signal absorbance to obtain normalized absorbance values.

Note: If more than 100 µL of medium is used per well, increase the amount of MTT Reagent accordingly; e.g., for 250 µL of medium use 25 µL of MTT Reagent.

Note: Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely a higher absorbance rate indicates an increase in cell proliferation. Rarely, an increase in proliferation may be offset by cell death; evidence of cell death may be inferred from morphological changes.

Note: Determine cytotoxicity of DMSO to the cells being tested. If DMSO is too toxic for accurate results, an alternative solution of 0.2% NP-40 and 8mM HCl in isopropanol can be used to solubilize the formazan. If this solution is used, include it as a blank.

Related Products

Product Name	Cat No
WST Plus-8, Cell Proliferation Assay Reagent	W0020
CellCount-Blue Cell Proliferation Assay Reagent	C0100
LucyQ Firefly Luciferase Assay Kit	L0010

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