Colorimetric Cell Viability Kit IV (MTT)

PromoKine

Instruction Manual

Product Name	Product Description	Size	Catalog Number
Colorimetric Cell Viability Kit IV (MTT)	Colorimetric Cell Viability Kit IV (MTT)	1000 Assays 200 Assays	PK-CA707-30006 PK-CA707-30006-200

Introduction

The PromoKine Cell Proliferation Assay Kit IV (MTT) provides a simple method for the determination of cell number using standard microplate absorbance readers. Determination of cell growth rates is widely used in the testing of drug action, cytotoxic agents and screening other biologically active compounds. Among a variety of non-radioactive cell proliferation assays, the MTT assay developed by Mossman (1) is still among one of the most versatile and popular assays.

The MTT assay is based on the cleavage of the yellow tetrazolium salt MTT to purple formazan crystal by metabolic active cells (2-4). The formazan is then solubilized, and the concentration determined by optical density at 570 nm. The result is a sensitive assay with a colorimetric signal proportional to the cell number. Promokine's MTT Cell Proliferation Assay Kit provides ready-to-use reagents for performing 1000 individual assays using standard 96-well microplates.

Kit Contents

PK-CA707-30006: 10 vials (1 ml each) with 1x MTT Solution

PK-CA707-30006-200: 2 vials (1 ml each) with 1x MTT Solution

Materials Required But Not Provided: Dimethylsulfoxide (DMSO)

Storage and Stability

Upon receipt, the kit should be stored at -20°C and protected from light. Stored properly, the kit components should remain stable for at least 6 months from date of receipt.

Cell Proliferation Assay Procedures

1. Plate cells into 96-well tissue culture plates. In general, cells should be seeded at densities between 5000 and 10,000 cells per well since they will reach optimal population densities within 48 to 72 hours.
2. Carry out desired cell treatment (e.g. adding your test substance such as chemicals or biological agents into appropriate well). The final volume of culture medium in each well should be 0.1ml, and the medium may contain up to 10% Fetal Bovine Serum.
3. Thaw one vial of MTT solution for each 96-well plate assay.

Note: If sediment is present in the solution, heat the solution to 37°C and swirl gently until a clear solution is obtained.

4. Add 10 µl MTT solution to 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. Note: At incubation times >2 hours MTT itself might have a certain but rather low cytotoxic effect on very sensitive cell types.
6. Add 200 µl DMSO 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 µl (a standard 96-well cell culture plate has a maximum volume of 400 µl).

7. Measure the absorbance signal on a spectrophotometer (e.g. an ELISA plate reader) at 570 nm. Measure background absorbance at 630 nm. Subtract background absorbance from signal absorbance (OD_{570}-OD_{630}) to obtain normalized absorbance values.

Intended Use

For in vitro research use only. Not for diagnostic or therapeutic procedures.

References

1. J Immunol Methods 65, 55 (1983);
2. J Neurochem 69, 581 (1997);
3. Arch Biochem Biophys 303, 474 (1993);
4. Cancer Res 51, 2515 (1991).

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