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Cell-Quant™ MTT Cell Proliferation Assay Kit
Catalog Number: A015

Table 1. Kit Components and Storage

Material	Amount	Storage	Stability
MTT Reagent (Component A)	**10 × 1 mL**	**-20 °C**	**The product is stable for at least six months when stored as directed.**
Detergent Reagent (Component B)	**100 mL**	**RT or 4 °C**	

Number of assays: 1000.

Note: Component A: dissolve 5 mg MTT in 1 mL of sterile PBS.
Component B: dissolve 1 g SDS in 10 mL of 0.01M HCl.

Introduction

Measurement of cell viability and proliferation forms the basis for numerous *in vitro* assays of a cell population's response to external factors. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means.
The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. The number of assay steps has been minimized as much as possible to expedite sample processing. The MTT Reagent yields low background absorbance values in the absence of cells. For each cell type the linear relationship between cell number and signal produced is established, thus allowing an accurate quantification of changes in the rate of cell proliferation.

Experimental Protocols

Note: The culture conditions used to grow the cells can affect the results and must be taken into consideration when analyzing the data. The age of the cultures, number of passages and details of the growth medium can all be important factors. The presence of phenol red in the final assay samples can seriously affect results. We strongly recommend that the cells be cultured in medium free of phenol red, if possible. Alternatively, the final incubation with the MTT can be performed after exchanging the cells into medium free of phenol red.

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 in order to reach optimal density within 48 to 72 hours.

2. For adherent cells, remove the medium and replace it with 100 µL of fresh culture medium. For non-adherent cells, centrifuge the microplate, pellet the cells, carefully remove as much medium as possible and replace it with 100 µL of fresh medium.

3. **(Optional)** Carry out desired cell treatment. The final volume of culture medium in each well should be 100 µL, and the medium may contain up to 10% Fetal Bovine Serum.

4. Add 10 μL **MTT Reagent** (Component A) to the 100 μL 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 shorted to 2 hours.

6. Add 100 μL **Detergent Reagent** (Component B) directly into the medium in each well and mix thoroughly using the pipette.

7. Incubate the microplate at 37°C for 2-4 hours or overnight in the dark.

8. Measure the absorbance signal in each well at 570 nm.