Cell Proliferation Kit (MTT)

***Storage*：** -20°C

MTT is stable over one year at 0-5℃ with protection from light. Store it at -20℃ for longer storage. Repeated thawing and freezing causes an increase in the background, which interferes with the assay. Please store the kit at 0-5oC for frequent use.

IT IS RECOMMENDED THAT THE ENTIRE PROTOCOL BE REVIEWED BEFORE STARTING THE ASSAY.

Product Description

Traditionally, the determination of cell growth is done by counting viable cells after staining with a vital dye. Alternative methods include measurement of radioisotope incorporation as a measure of DNA synthesis, automated cell counters and other techniques which rely on dyes and cellular activity. The MTT system is a simple, accurate, reproducible means of measuring the activity of living cells via mitochondrial dehydrogenase activity. The key component is 3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyl tetrazolium bromide or MTT. Solutions of MTT solubilized in tissue culture media or balanced salt solutions, without phenol red, are yellowish in color. Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, yielding purple MTT formazan crystals which are insoluble in aqueous solutions. The crystals can be dissolved in acidified isopropanol. The resulting purple solution is spectrophotometrically measured. An increase in cell number results in an increase in the amount of MTT formazan formed and an increase in absorbance. The use of the MTT method does have limitations influenced by: (1) the physiological state of cells and (2) variance in mitochondrial dehydrogenase activity in different cell types. Nevertheless, the MTT method of cell determination is useful in the measurement of cell growth in response to mitogens, antigenic stimuli, growth factors and other cell growth promoting reagents, cytotoxicity studies, and in the derivation of cell growth curves.

Protocol：

The MTT method of cell determination is most useful when cultures are prepared in multiwell plates. For best results, cell numbers should be determined during log growth stage. Each test should include a blank containing complete culture medium without cells.

NOTE: Bacteria, mycoplasma and other microbial contaminants may also cleave the MTT tetrazolium ring; thus contaminated cultures should not be tested by this method.

1. Remove cultures from incubator into laminar flow hood or other sterile working area.
2. Aseptically add MTT SOLUTION in an amount equal to 10% of the culture volume.

3. Return cultures to incubator and incubate for 3 to 4 hours. Incubation times should be consistent when making comparisons.
4. After the incubation period, remove cultures from incubator and dissolve the resulting MTT formazan crystals as follows:

a. If cells are attached to culture vessel growth surface, remove and dispose of the culture fluid. Add MTT SOLVENT in an amount equal to the original culture volume. Solvent volumes may vary but the final volumes should be consistent to facilitate comparison.

b. If cells are not attached or loss of MTT formazan occurs if culture fluid is removed, add MTT SOLVENT directly to the culture in an amount equal to the original culture volume.

c. Plates should be read within 1 hour after adding MTT SOLVENT.

5. Gentle stirring in a gyratory shaker will enhance dissolution.Occassionally, pipetting up and down (trituration) may be required to completely dissolve the MTT formazan crystals especially in dense cultures.
6. Spectrophotometrically measure absorbance at a wavelength of 570 nm. Subtract background absorbance measured at 690 nm.
7. Tests performed in 96 well plates may be measured in an ELISA- type plate reader equipped with appropriate filters.
8. Tests performed in other multiwell plates will require transfer to appropriate size cuvets or plate reader for spectrophotometric measurement.

NOTE: MTT conversion to MTT formazan is cell-type specific. Because of variability that may occur between different strains of the same cell line and because of the influence of the physiological state of the cells, it is recommended that researchers prepare their own absorbance/cell number curves.

POSSIBLE SOURCES OF ERROR

1. MTT SOLUTION is stable when stored frozen. Storage at 2-8 °C may result in decomposition and yield erroneous results. Development of dark color or formation of crystals indicate product deterioration.
2. Microbial contamination will contribute to the cleavage of MTT and formation of MTT formazan yielding erroneous results.
3. Uneven evaporation of culture fluid in wells of multiwell plates may cause erroneous results.
4. High protein levels (serum, albumin, etc.) in the culture medium may form a precipitate when MTT SOLVENT is added. Samples with protein concentrations equivalent to 10% fetal bovine serum seem acceptable. Sera with higher protein concentrations than fetal bovine serum may have to be used at lower percentages.