Cell Proliferation Kit (MTS)

Storage： -20°C

MTS kit is stable 6 week at 4°C with protection from light. Store it at -20°C for longer storage. Repeated thawing and freezing causes an increase in the background, which interferes with the assay. Please store the kit at 0-5°C for frequent use.

Introduction：

The Cell Proliferation Kit (MTS) kit is a colorimetric method for determining the number of viable cells in proliferation or cytotoxicity assays. The *MTS kit* One Solution Reagent contains a novel tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS(a)] and an electron coupling reagent (phenazine ethosulfate; PES). PES has enhanced chemical stability, which allows it to be combined with MTS to form a stable solution. This convenient "One Solution" format is an improvement over the fist version of the MTS kit Assay, where phenazine methosulfate (PMS) is used as the electron coupling reagent, and the PMS Solution and MTS Solution are supplied separately. The MTS tetrazolium compound (Owen's reagent) is bioreduced by cells into a colored formazan product that is soluble in tissue culture medium. This conversion is presumably accomplished by NADPH or NADH produced by dehydrogenase enzymes in metabolically active cells. Assays are performed by adding a small amount of the MTS kit One Solution Reagent directly to culture wells, incubating for 1–4 hours and then recording the absorbance at 490nm with a 96-well plate reader.

Protocol：

1. Thaw the MTS kit One Solution Reagent. It should take approximately 90 minutes at room temperature, or 10 minutes in a water bath at 37°C, to completely thaw the total size.

2. Pipet 10µl of MTS kit One Solution Reagent into each well of the 96-well assay plate containing the samples in 100µl of culture medium.

Note: We recommend repeating pipettes, digital pipettes or multichannel pipettes for convenient delivery of uniform volumes of MTS kit One Solution Reagent to the 96-well plate.

3. Incubate the plate at 37°C for 1–4 hours in a humidifi ed, 5% CO2 atmosphere.

Note: To measure the amount of soluble formazan produced by cellular reduction of MTS, proceed immediately to Step 4. Alternatively, to measure the absorbance later, add 25µl of 10% SDS to each well to stop the reaction. Store SDS-treated plates protected from light in a humidifi ed chamber at room temperature for up to 18 hours. Proceed to Step 4.

4. Record the absorbance at 490nm using a 96-well plate reader.

Notes

1. How many cells should there be in a well?

Cell proliferation assays require cells to grow over a period of time. Therefore, choose an initial number of cells per well that produces an assay signal near the low end of the linear range of the assay. This helps to ensure that the signal measured at the end of the assay will not exceed the linear range of the assay. This cell number can be determined by performing a cell titration.Different cell types have different levels of metabolic activity. Factors that affect the metabolic activity of cells may affectthe relationship between cell number and absorbance. Anchorage-dependent cells that undergo contact inhibition may show a change in metabolic activity per cell at high densities, resulting in a nonlinear relationship between cell number and absorbance. Factors that aff ect the cytoplasmic volume or physiology of the cells will aff ect metabolic activity. For most tumor cells, hybridomas and fi broblast cell lines, 5,000 cells per well is recommended to initiate proliferation studies, although fewer than 1,000 cells can usually be detected. The known exception to this is blood lymphocytes,

which generally require 25,000–250,000 cells per well to obtain a suffi cient absorbance reading.

2. Background Absorbance

A small amount of spontaneous 490nm absorbance occurs in culture medium incubated with MTS kit One Solution Reagent. The type of culture medium used, type of serum, pH and length of exposure to light are variables that may contribute to the background 490nm absorbance. Background absorbance is typically 0.2–0.3 absorbance units after 4 hours of culture. Background absorbance may result from chemical interference of certain compounds with tetrazolium reduction reactions. Strong reducing substances, including ascorbic acid, or sulfhydryl-containing compounds, such as glutathione, coenzyme A and dithiothreitol, can reduce tetrazolium salts nonenzymatically and lead to increased background absorbance values. Culture medium at elevated pH or extended exposure to direct light also may cause an accelerated spontaneous reduction of tetrazolium salts and result in increased background absorbance values. If phenol red containing medium is used, an immediate change in color may indicate a shift in pH caused by the test compounds. Specifi c chemical interference of test compounds can be confi rmed by measuring absorbance values from control wells containing medium without cells at various concentrations of test compound.

Background 490nm absorbance may be corrected as follows: Prepare a triplicate set of control wells (without cells) containing the same volumes of culture medium and CellTiter 96? AQueous One Solution Reagent as in the experimental wells. Subtract the average 490nm absorbance from the "no cell" control wells from all other absorbance values to yield corrected absorbances.