MTT Cell Proliferation Assay

Cell Biology

CATALOGUE NO.: CTK003

DESCRIPTION: A colorimetric assay for measuring the activity of enzymes that reduce MTT to formazan, giving a purple color. 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. A solubilization solution is used to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer. The absorption maximum is dependent on the solvent employed. 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 MTT Cell Proliferation Assay measures the cell proliferation rate and 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. Conversely it is widely used to determine cytotoxicity of external treatments, 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.

KIT COMPONENTS:

MTT Reagent	10 ml
Solvent	100 ml

STORAGE: Stable for >1year at 4-8°C.

PACKING SIZE: 1 kit (1000 standard reactions in 96-well plate).

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents & Consumables:

Phosphate Buffered Saline (PBS), sterile

Cell culture medium, sterile

96-well plate(s), tissue culture grade, flat bottomed, sterile

5 ml tubes, sterile

Serological pipettes, sterile

Pipette tips (1 – 200 ul), sterile

Equipment:

Inverted microscope

Multichannel pipettor

Cell culture facilities including a laminar flow hood and a 37° C CO2 incubator

Plate reader with 650 and 570 nm filters

EXPERIMENTAL PROCEDURE:

1. Plate cells at the confluence determined using the procedure in Appendix section. Plate triplicate wells at 100 uL/well for each variable. Be sure to plate enough wells to include cell-based controls, and include three wells of cell culture medium alone for normalization purpose.
2. Incubate the cells to allow them to recover and reattach (if adherent) and treat according to your established experimental protocol.
3. Add 10 uL of MTT reagent to each well. If more than 100 uL of cell culture medium was used per well, increase the amount of MTT added proportionately.
4. Incubate the plate for approximately 2-4 hours at 37°C. View the cells periodically for the appearance of punctate, intracellular precipitate using an inverted microscope. Longer incubation times (up to 24 hours) may be required, depending on the cell type and experimental conditions.
5. When purple precipitate is clearly visible under the microscope, add 100 uL of Solvent Reagent to all wells, including control wells. Do not shake.
6. Leave plate covered in the dark at 18-24°C for at least 2 hours to overnight. Samples may be read after 2 hours, but if the readings are low and there are crystals remaining, return the plate to the dark and incubate for a longer period. Room temperature (18-24°C) incubation is sufficient, but incubation at 37° C may help to shorten the solubilization time.
7. Remove the plate cover and measure the absorbance of the wells, including the blanks, at 570 nm with a reference wavelength of 650 nm. If a 570 nm filter is not available, absorbances may be read with any filter in the wavelength range of 550-600 nm.
8. Determine the average values from triplicate readings and subtract the average value for the blank. Plot absorbance on the y-axis versus treatment on the x-axis.

Note:

- ✧ Negative controls must be run in the assay to be sure that it is functioning correctly.
  The absorbance value for the blanks should be 0±0.1 OD units.
  The absorbance range for untreated cells should typically be between 0.75 and 1.25 O.D. units.
- ✧ Positive controls established for your type of cells should also be run.

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