MTT Cell Proliferation Assay

ATCC® 30-1010K
Store at 4°C

This product is intended for laboratory research purposes only. It is not intended for use in humans, animals or for diagnostics.

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.

KIT COMPONENTS

Component	Volume	Storage
MTT Reagent	25 mL	4°C
Detergent Reagent	2 × 125 mL	Room Temp. or 4°C

The MTT Reagent is ready to use and stable at 4°C in the dark for up to eighteen months, provided there is no contamination. Care should be taken not to contaminate the MTT Reagent with cell culture medium during pipetting. We recommend that the appropriate volume required for each experiment be removed and aseptically placed into a separate clean tube and the stock bottle returned to 4°C in the dark. If the MTT Reagent is bluegreen, do not use and refer to the troubleshooting guide on page 6.

The Detergent Reagent is supplied ready to use. If the Detergent Reagent has been stored at 4°C, warm the bottle for 5 minutes at 37°C then mix by inverting gently to avoid frothing. The detergent is stable for up to eighteen months at room temperature.

EQUIPMENT AND MATERIALS REQUIRED BUT *NOT* SUPPLIED

Microtiter plate reader with 650- and 570-nm filters	Microtiter plate (flat-bottomed)
Inverted microscope	Sterile tubes (5 mL)
Multi-channel pipette	Serological pipettes
37°C incubator	Sterile pipette tips
Laminar flow hood	

BASIC PROTOCOL

If you are familiar with the procedure and know the cell count to use in your specific assay, you may follow this basic protocol.

Step	Action
1	Plate cells at 1,000 to 100,000 per well.
2	Incubate for 6 to 24 hours.
3	Add 10 µL MTT Reagent.
4	Incubate for 2 to 4 hours until purple precipitate is visible.
5	Add 100 µL Detergent Reagent.
6	Leave at room temperature in the dark for 2 hours.
7	Record absorbance at 570 nm.

DETERMINING OPTIMAL CELL COUNTS

Use the protocol below to determine the optimal cell count and incubation period for your cell line. This determination should only have to be done once for each cell type. The data will be used thereafter in your experimental system following the protocol above.

Step	Action
1	Harvest suspension cells by centrifugation. Adherent cells should be released from their substrate by trypsinization or scraping.
2	Resuspend cells at 1 x 10^6 per mL.
3	Prepare serial dilutions of cells in culture medium from 1 x 10^6 to 1 x 10^3 cells per mL.
4	Plate out, in triplicate, 100 µL of the dilutions into wells of a microtiter plate.
5	Include three control wells of medium alone to provide the blanks for absorbance readings.
6	Incubate the cells under conditions appropriate for the cell line for 6 to 48 hours (to recover from handling). The time required will vary but 12 hours to overnight is sufficient for most cell types.
7	Add 10 µL of MTT Reagent to each well, including controls.
8	Return plate to cell culture incubator for 2 to 4 hours.
9	Periodically view the cells under an inverted microscope for presence of intracellular punctate purple precipitate.
10	When the purple precipitate is clearly visible under the microscope add 100 µL of Detergent Reagent to all wells, including controls. Swirl gently; do not shake.
11	Leave plate with cover in the dark for 2 to 4 hours or overnight at room temperature.
12	Remove plate cover and measure the absorbance in each well, including the blanks, at 570 nm in a microtiter plate reader. [Absorbances can be read with any filter in the wavelength range of 550 - 600 nm. The reference wavelength should be higher than 650 nm. The blanks should give values close to zero (+/- 0.1).]
13	If the readings are low return the plate to the dark for longer incubation.
14	Determine the average values from triplicate readings and subtract the average value for the blank. Plot absorbance against number of cells/mL. The number of cells to use in your assay should lie within the linear portion of the plot and yield an absorbance of 0.75 - 1.25.

PERFORMING AN ASSAY

The plot of the data obtained in Step 14 on page 3 (absorbance against number of cells) should provide a curve with a linear portion. The optimal number of cells for the assay should fall within the linear portion of the curve and give an absorbance value between 0.75 and 1.25. Then both stimulation and inhibition of cell proliferation can be measured.

To run an assay, select an optimal cell number and follow the MTT Cell Proliferation Assay steps 4 to 13 (page 3) using your experimental system, plating in triplicate. Assays will include:

a) Blank wells containing medium only
b) Untreated control cells
c) Test cells treated with the substance to be assayed

If more than 100 µL of medium is used per well, increase the amount of MTT Reagent accordingly; e.g., for 250 µL of medium use 25 µL of MTT Reagent.

DATA INTERPRETATION

Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely a higher absorbance rate indicates an increase in cell proliferation. Rarely, an increase in proliferation may be offset by cell death; evidence of cell death may be inferred from morphological changes.

REFERENCES

van de Loosdrecht, A.A., et al. J. Immunol. Methods 174: 311-320, 1994.
Ferrari, M., et al. J. Immunol. Methods 131: 165-172, 1990.
Gerlier, D., and N. Thomasset. J. Immunol. Methods 94: 57-63, 1986.
Alley, M.C., et al. Cancer Res. 48: 589-601, 1988.
Mosmann, T. J. Immunol. Methods 65: 55-63, 1983.

TROUBLESHOOTING

Problem: MTT Reagent is blue-green.

Cause	Remedy
Contamination with a reducing agent or cell/bacterial contamination.	Discard. Remove aliquots of new MTT Reagent using sterile technique.
Excessive exposure to light.	Store solution in the dark at 4°C.

TROUBLESHOOTING (CONTINUED)

Problem: Blanks (medium only) give high absorbance readings.

Cause	Remedy
The medium is contaminated with cells/ bacteria/yeast (visible under microscope).	Discard. Check medium before plating. Use sterile technique for cell plating in biological hood. Use sterile 96-well plate.
The medium contains ascorbic acid.	Incubate plate in the dark. Find alternative medium if possible.

Problem: Absorbance readings too high.

Cause	Remedy
Cell number per well too high.	Decrease cell density at plating.
Contamination of culture with bacteria or yeast.	Discard. View wells prior to addition of MTT Reagent to check for contamination.

Problem: Absorbance readings are too low.

Cause	Remedy
Cell number per well is too low.	Increase cell density at plating.
Incubation time for reduction of MTT is too short. No purple color visible in cells when viewed under microscope.	Increase incubation time with MTT Reagent until purple color is evident inside cells when viewed under microscope. Longer incubation of up to 24 hours may be required for some cell types.
Incubation time for solubilization of formazan dye too short (intact cells with intracellular dye visible when viewed under the microscope).	Increase incubation time with Detergent Reagent or incubate at 37°C. View under microscope to ensure no crystals remain out of solution.
Cells not proliferating due to improper culture conditions or inadequate time of recovery after plating.	Check that culture conditions (medium, temperature, humidity, CO_2, etc.) are appropriate. View cells periodically to check condition. Increase time in culture after plating for cell recovery.

Problem: Replicates have different values.

Cause	Remedy
Inaccurate plating or pipetting.	Increase accuracy of cell plating, check accuracy of pipette.

SAFETY

See the Material Safety Data Sheet regarding safety precautions for this product.

RELATED PRODUCTS

ATCC has the world's largest collection of cell lines. If you need a cell line as a control for performing your assay, see our Web site at www.atcc.org to search our online catalog. We also offer cell culture media, serum, and reagents.

American Type Culture Collection
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