TREVIGEN® Instructions

For Research Use Only. Not For Use In Diagnostic Procedures

TACS® MTT
Cell Proliferation Assays

Cat# 4890-25-K, 2500 Tests
Cat# 4890-50-K, 5000 Tests

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Table of Contents

I. Background

Measurement of cell viability and proliferation comprise the underlying basis for numerous *in vitro* assays directed towards the quantitation of a cell population's response to external factors. Cell proliferation assays have utilized the uptake of radiolabeled thymidine into cellular DNA, however, the method is time consuming and involves the use of hazardous materials. An alternative method is provided by the reduction of tetrazolium salts which is now widely accepted as a reliable method for examining cell proliferation. The yellow tetrazolium salt 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl-**tetrazolium** bromide (MTT) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resultant intracellular purple formazan can be solubilized and quantitated by spectroscopic means.

Trevigen's **TACS® MTT Cell Proliferation Assay** allows measurement of proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. The MTT Cell Proliferation Kit minimizes the number of steps necessary to complete the assay and interpret the data. The MTT reagent yields low background absorbance values in the absence of cells and is stable when stored at 4 ºC. For each cell type the linear relationship between cell number and signal produced is established thus allowing an accurate quantitation of changes in the rate of cell proliferation.

II. Precautions and Limitations

1. For Research Use Only. Not for use in diagnostic procedures.

2. The physical, chemical and toxicological properties of the provided products may not yet have been fully investigated; therefore, Trevigen recommends the use of gloves, lab coats, and eye protection while using these chemical reagents. Trevigen assumes no liability for damage resulting from handling or contact with these products.

3. MTT Reagent (Cat# 4890-25-01) contains less than 1% (w/v) MTT (3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyl-tetrazolium bromide (CAS # 298-93-1). MTT is toxic and may cause heritable genetic defects. In case of contact, immediately flush eyes or skin with copious amounts of water. If swallowed, wash out mouth with water provided person is conscious. Call a physician.

4. Detergent Solution (Cat# 4890-25-02) contains SDS which is an irritant. In case of contact, immediately flush eyes or skin with copious amounts of water. If swallowed, wash out mouth with water provided person is conscious.

III. Materials Supplied

Cat# 4890-25-K

Component	Quantity	Storage	Catalog #
MTT Reagent	25 ml	4 °C	4890-25-01
Detergent Reagent	250 ml	room temp	4890-25-02

Cat# 4890-50-K

Component	Quantity	Storage	Catalog #
MTT Reagent	2 x 25 ml	4 °C	4890-25-01
Detergent Reagent	2 x 250 ml	room temp	4890-25-02

IV. Materials/Equipment Required But Not Supplied

Equipment

1. microplate plate reader: 650 and 570 nm filters
2. inverted microscope
3. multichannel pipette
4. pipette aid
5. 37 °C incubator
6. laminar flow hood

Reagents

1. cell culture medium
2. microplate plate (flat bottomed)
3. sterile tubes (5 ml)
4. serological pipettes
5. sterile pipette tips

V. Reagent Preparation

1. **MTT Reagent**
   The MTT Reagent (Cat# 4890-25-01) is supplied ready for use. The MTT Reagent is stable at 4 °C provided there is no contamination. Care should be taken not to contaminate the MTT reagent with cell culture medium during pipetting. It is recommended that the appropriate volume required for each experiment is aliquoted and placed into a separate clean tube under sterile conditions and the stock bottle is returned to 4 °C in the dark. If the MTT Reagent is blue-green in color do not use and refer to the Troubleshooting Guide on page 6.

2. **Detergent Reagent**
   The Detergent Solution (Cat# 4890-25-02) is supplied ready for use. If the Detergent Reagent has been stored at 4 °C, warm the bottle for 5 minutes at 37 °C then invert gently while mixing to avoid frothing.

VI. Assay Protocol

Cells are cultured in 100 µl of culture medium in a flat-bottomed 96 well plate (tissue culture grade). The incubation period and the cell plating density should be determined for each cell type and the experimental conditions. The MTT reagent is added (10 µl per well) and the plate is incubated for 2 to 12 hours to allow for intracellular reduction of the soluble yellow MTT to the insoluble purple formazan dye. Detergent reagent is added to each well to solubilize the formazan dye prior to measuring the absorbance of each sample in a microplate reader at 550 - 600 nm, depending upon the filters available. The complete protocol for optimizing the assay for your experimental system is given below.

Step	Instructions	Notes
1	Resuspend cells at 1 x 10^6 per ml	Harvest suspension cells by centrifugation. Adherent cells should be released from their substrate by trypsinization or scraping.
2	Prepare dilutions of cells from 1 x 10^6 to 1 x 10^4 cells per ml in order to plate cells at 10^3 – 10^5 per well.	The number of cells per well required for optimal results will vary depending upon cell type, culture conditions, etc.
3	Distribute, in triplicate, 100 µl of the dilutions per well. Include three controls of medium alone.	Cells with medium alone provides the blank for the absorbance readings.
4	Incubate the cells for 6 to 48 hours.	The cells need time to recover and/or adhere to the substrate. The time required will vary between cell types but 2 hours to overnight is sufficient for most cell lines.
5	Add 10 µl of MTT reagent (Cat# 4890-25-01) to each well.	If more than 100 µl of medium was used per well increase the amount of MTT Reagent used accordingly e. g. for 250 µl of medium use 25 µl of MTT reagent. To avoid contamination of the MTT reagent it is advisable to place a sample of MTT Reagent in a clean tube for aliquots and return the stock solution to 4 °C in the dark.
6	Return plate to cell culture incubator for 2 to 4 hours until purple dye is visible.	Periodically view the cells under an inverted microscope for presence of intracellular punctate purple precipitate. Longer periods of incubation of up to 24 hours may be required for some cell types.

VI. Assay Protocol (continued)

Step	Instructions	Notes
7	When the purple precipitate is clearly visible under the microscope, add 100 µl of Detergent Reagent (Cat# 4890-25-02) to all wells. Do not shake.	Samples can be read after 2 hours. If the readings are low return the plate to the dark and incubate for a longer period. The solubilization time may be shortened by incubation at 37 ^{o}C but room temperature is usually adequate.
8	Leave plate with cover in the dark for 2 to 4 hours or overnight at room temperature.	Samples can be read after 2 hours. If the readings are low return the plate to the dark and incubate for a longer period. The solubilization time may be shortened by incubation at 37 ^{o}C but room temperature is usually adequate.
9	Remove plate cover and measure the absorbance in each well, including the blanks, at 570 nm in a microplate 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).
10	Determine the average values from triplicate readings and subtract the average value for the blank. Plot absorbance against cell number/ml. Select a cell number that yields an absorbance of 0.75 - 1.25.	The cell number selected should lie within the linear portion of the plot.
11	Analyze the experimental system to be tested using the cell number per well determined in step 10 above, in triplicate, and repeat MTT Cell Proliferation Assay steps 3 to 9.	Perform appropriate controls including blanks (media only) and untreated cells (refer to section VII).

VII. Controls

The minimum number of controls that should be included when running your assay are:

1. Blank wells containing medium only.

2. Untreated control cells. The absorbance range for the control cells (i.e. untreated) should typically be between 0.75 and 1.25. The cell number for plating should be determined using the procedure described in the Assay Protocol (page 3).

VIII. Data Interpretation and Troubleshooting

Problem	Cause	Solution
MTT Reagent is blue/green.	Contamination with a reducing agent or cell/bacterial contamination.	Discard, remove aliquots of new MTT reagent using sterile procedure.
	Excessive exposure to light.	Store solution in the dark at 4 ^{o}C.
Blanks (media only) give high absorbance readings.	The media is contaminated with cells/bacteria/yeast (visible under microscope).	Discard. Check media solution before plating. Use sterile technique for cell plating in biological hood. Use sterile 96 well plate.
	The media contains ascorbic acid.	Find alternative medium if possible. Incubate plate in the dark.
Absorbance readings are too low.	Cell number per well is too low.	Increase cell density at plating.
	Incubation time for reduction of MTT intracellularly too short. No purple color in cells visible when viewed under microscope.	Increase incubation time with MTT reagent until purple color evident inside cells when viewed under microscope.
	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. 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 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	Cause	Solution
Absorbance readings too high	Cell number per well too high. Contamination of culture with bacteria, yeast.	Decrease cell density at Plating. Discard. View wells prior to addition of MTT reagent to check for contamination.
Replicates have different values	Innacurate plating or pipetting.	Increase accuracy of cell plating, check accuracy of pipette.

IX. References

1. van de Loosdrecht, A.A., R.H. Beelen, G.J. Ossenkoppele, M.G. Brockhoven, and M.M. Langenhuijsen (1994) A tetrazolium-based colorimetric MTT assay to quantitate human monocyte mediated cytotoxicity against leukemic cells from cell lines and patients with acute myeloid leukemia. J. Immunol. Methods **174**:311-20.

2. Alley, M.C., D.A. Scudiero, A. Monks, M.L. Hursey, M.J. Czerwinski, D.L. Fine, B.J. Abbott, J.G. Mayo, R.H. Shoemaker, and M.R. Boyd (1988) Feasibility of drug screening with panels of human tumor cell lines using microculture tetrazolium assay. Cancer Res. **48**:589-601.

3. T. Mosmann (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays J. Immunol. Methods **65**:55-3.

X. Related Products

Catalog #	Description	Size
4891-025-K	**TACS® XTT Cell Proliferation Assay**	**2500 tests**
4892-010-K	**Cultrex® Calcein-AM Cell Viability Kit**	**1000 tests**
4817-60-K	**FlowTACS™ Apoptosis Detection Kit**	**60 samples**
4822-96-K	**HT TiterTACS™ Assay Kit**	**96 tests**
4830-01-K	**TACS® Annexin V FITC Kit**	**100 samples**
4835-01-K	**TACS® Annexin V Biotin Kit**	**100 samples**
6300-100-K	**DePsipher™ Mitochondrial Potential Assay Kit**	**100 tests**
6305-100-K	**MitoShift™ Mitochondrial Potential Assay Kit**	**100 tests**
3445-096-K	**Cultrex® 3D Culture BME Cell Proliferation Assay Kit**	**96 tests**

The product accompanying this document is intended for research use only and is not intended for diagnostic purposes or for use in humans.

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