TACS™ MTT Assays

Cell Proliferation and Viability Assays

Catalog Number: TA5355 - 2500 tests

Catalog Number: TA5412 - 5000 tests

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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PRINCIPLE OF THE ASSAY

The TACS MTT Cell Proliferation and Viability Assay is a safe, sensitive, *in vitro* assay for the measurement of cell proliferation or, when metabolic events lead to apoptosis or necrosis, a reduction in cell viability. Cells are cultured in flat-bottomed, 96-well tissue culture plates. The cells are treated as per experimental design and incubation times are optimized for each cell type and system. The tetrazolium compound MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) is added to the wells and the cells are incubated. MTT is reduced by metabolically active cells to insoluble purple formazan dye crystals. Detergent is then added to the wells, solubilizing the crystals so the absorbance can be read using a spectrophotometer. Samples are read directly in the wells. The optimal wavelength for absorbance is 570 nm, but any filter that absorbs between 550 and 600 nm may be used. The data is analyzed by plotting cell number versus absorbance, allowing quantitation of changes in cell proliferation. The rate of tetrazolium reduction is proportional to the rate of cell proliferation.

REAGENTS PROVIDED

Component #	Component	Quantity	Storage Conditions
4890-25-01	MTT Reagent	25 mL (2500 tests) 2 x 25 mL (5000 tests)	2 - 8° C
4890-25-02	Detergent Reagent	250 mL (2500 tests) 2 x 250 mL (5000 tests)	18 - 24° C

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

- 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 μL), sterile

Equipment

- Inverted microscope
- Multichannel pipettor
- Cell culture facilities including a laminar flow hood and a 37° C CO_2 incubator
- Plate reader with 650 and 570 nm filters

REAGENT HANDLING INFORMATION

MTT Reagent

The MTT reagent is ready for use. To prevent contamination, aseptically remove the appropriate volume for use during the entire experiment and place it into a separate tube. Return the remainder to storage at 2 - 8° C in the dark*. Contamination will compromise the stability of this reagent. Be careful not to get any tissue culture medium into the MTT stock solution. Do not use if blue/green in color (refer to the Troubleshooting Guide on page 6).*

Detergent Solution

The Detergent Solution is ready for use. It is stored at 18 - 24° C. If the solution becomes cold, warm at 37° C for 5 minutes. Invert gently to mix, taking care not to induce foaming.

PROCEDURES

Follow the section below, to determine the optimal cell number to be used in your system.
Note: *This should only have to be performed once for each cell type. Proceed to MTT Assay for Experimental Samples section (page 4) for instructions on how to run an assay using your experimental parameters.*

MTT Assay for Determination of Cell Number to be Used

1. Harvest cells. Suspension cells may be harvested by centrifugation at 500 x g for 5 minutes at 2 - 8° C. Adherent cells should be released from their substrate by trypsinization or scraping. Pellet cells by centrifugation at 500 x g for 5 minutes at 2 - 8° C. Discard supernate. *The centrifugation speed may have to be adjusted for individual cell types.*
2. Wash cells by resuspending in 5 mL sterile PBS or cell culture medium. Pellet cells by centrifugation at 500 x g for 5 minutes at 2 - 8° C. Discard supernate.
3. Resuspend cells at 5 x 10^6 cells/mL in tissue culture medium. Harvest and wash sufficient cells to prepare 8 - 10 serial two-fold dilutions with 100 µL of cells/well, in triplicate.
4. Serially dilute cells using 5 mL culture tubes. Dilutions from 5 x 10^6 to 5 x 10^3 cells/mL should be sufficient for most cell types. For example, ten 2-fold dilutions from 5 x 10^6 cells/mL will result in concentrations from 2.5 x 10^6 to 4.88 x 10^3 cells/mL (see table below). For this example, you will need 0.8 mL of cells at 5 x 10^6 cells/mL for a total of 4 x 10^6 cells. Refer to the table.

Label Tubes (cells/mL)	Add Cell Culture Medium	Add Cells
5.00 x 10^6	—	400 µL of 5.00 x 10^6 cells/mL stock
2.50 x 10^6	400 µL	400 µL of 5.00 x 10^6 cells/mL stock
1.25 x 10^6	400 µL	400 µL of 2.50 x 10^6 cells/mL stock
6.25 x 10^5	400 µL	400 µL of 1.25 x 10^6 cells/mL stock
3.13 x 10^5	400 µL	400 µL of 6.25 x 10^5 cells/mL stock
1.56 x 10^5	400 µL	400 µL of 3.13 x 10^5 cells/mL stock
7.81 x 10^4	400 µL	400 µL of 1.56 x 10^5 cells/mL stock
3.91 x 10^4	400 µL	400 µL of 7.81 x 10^4 cells/mL stock
1.95 x 10^4	400 µL	400 µL of 3.91 x 10^4 cells/mL stock
9.77 x 10^3	400 µL	400 µL of 1.95 x 10^4 cells/mL stock
4.88 x 10^3	400 µL	400 µL of 9.77 x 10^3 cells/mL stock
Medium Control	400 µL	—

5. Plate cells at 100 µL/well. Include 3 control wells of cell culture medium alone (see section on controls, page 5).
6. Incubate the cells for 6 - 48 hours. Cells need time to recover and reattach (if adherent). This will vary for each cell type. In general, 12 - 18 hours is sufficient.
7. Add 10 µL of MTT reagent to each well.
8. Incubate the plate for 2 - 4 hours at 37° C. View the cells periodically for the appearance of punctate, intracellular precipitate using an inverted microscope. Some cell types may require a longer incubation, as much as 24 hours.
9. When purple precipitate is clearly visible under the microscope, add 100 µL of Detergent Reagent to all wells, including control wells. **Do not shake**.
10. Leave covered plate 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 shorten the solubilization time.
11. 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. The blanks should give values of 0 ± 0.1 O.D. units*.
12. Determine the average values from triplicate readings and subtract the average value for the blank. Plot absorbance on the y-axis versus cell number per mL on the x-axis. Select a cell number that yields an absorbance of 0.75 to 1.25. The cell number selected should fall within the linear portion of the curve.

MTT Assay for Experimental Samples

1. Plate cells at the concentration determined using the procedure on pages 3 - 4. Plate triplicate wells at 100 µL/well for each variable. Be sure to plate enough wells to include cell-based controls, and include three wells of cell culture medium alone (see section on controls, page 5).
2. Incubate the cells to allow them to recover and reattach (if adherent) and treat according to your established experimental protocol.
3. Add 10 µL of MTT reagent to each well. If more than 100 µL of cell culture medium was used per well, increase the amount of MTT added proportionately.
   Note: *Transfer the full amount required for the entire experiment to a separate tube and return the stock to storage at 2 - 8° C in the dark. Do not take multiple aliquots from the MTT stock for each experiment.*
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 µL of Detergent 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. The blanks should give values of 0 ± 0.1 O.D. units.*
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.

CONTROLS

Note: *These controls must be run in the assay to be sure that it is functioning correctly. Positive controls established for your type of cells should also be run.*

- The absorbance value for the blanks should be 0.00 ± 0.1 OD units.
- The absorbance range for untreated cells should typically be between 0.75 and 1.25 O.D. units.

DATA INTERPRETATION

The plot of data obtained from the procedure on page 3 (MTT Assay for Determination of Cell Number to be Used) should provide a curve that has a linear portion. Selection of a cell number that falls within the linear portion of the curve (*i.e.* providing values between the range of 0.75 and 1.25) allows for the measurement of both stimulation and inhibition of cell proliferation.

If the absorbance values of the experimental samples are higher than the negative control cells, this indicates an increase in cell proliferation. Alternatively, if the absorbance rates of the experimental samples are lower than the negative controls, this indicates a reduction in the rate of cell proliferation or a reduction in overall cell viability.

In some rare instances, an increase in cell proliferation may be offset by cell death. Programmed cell death may be determined by observation of apoptosis *in situ* and confirmed by DNA laddering.

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
MTT Reagent is blue/green.	Cellular or bacterial contamination. Contamination with a reducing agent. Excessive exposure to light.	Discard. Use sterile procedure to remove aliquots from a new vial of MTT. Remove amount necessary for one experiment into a fresh tube and return the remainder of the MTT stock to storage at 2 - 8° C in the dark.
Blanks (media only) give high absorbance readings.	Cell culture medium is contaminated with bacteria, cells or yeast. Cell culture medium contains ascorbic acid.	Discard. Check medium before using. Use sterile technique in a laminar flow hood to plate the cells. Use sterile 96 well plates. Find alternative medium, if possible. Incubate the plate in the dark.
Absorbance readings are too low.	Cells are not proliferating due to experimental protocol, improper culture conditions or inadequate cell recovery time after plating.	Check/optimize experimental conditions, culture conditions (medium, incubator temperature, humidity and CO_2 level). Increase cell recovery time after plating.
Absorbance readings are too high.	Cell number too high. Contamination of cell cultures with bacteria or yeast.	Decrease cell plating density. Discard. Use new medium and reagents or check old ones for contamination. View cells for presence of contamination prior to addition of MTT.
Replicates have poor CVs (replicates vary).	Inaccurate plating or pipetting. Contamination of cell cultures with bacteria or yeast.	Increase accuracy of cell plating. Check accuracy of pipettors. Discard. Use new medium and reagents or check old ones for contamination. View cells for presence of contamination prior to addition of MTT.

WARNINGS

Handling Precautions

Safe laboratory procedures should be followed when handling all kit reagents. It is recommended that protective laboratory clothing and equipment (gloves, laboratory coat, safety glasses) be worn when handling kit reagents.

Emergency Exposure Procedures

In case of exposure to reagent solutions, we recommend following these emergency first-aid procedures:

Skin or eye contact
Wash with water for at least 15 minutes. Remove any contaminated clothing.

Inhalation
Remove individual to fresh air. If breathing is difficult, give oxygen and call a physician.

Ingestion
Rinse mouth with copious amounts of water and call a physician.