R&D SYSTEMS
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TACS® MTT Cell Proliferation Assay

Catalog Numbers: 4890-025-K (2500 Tests)
4890-050-K (5000 Tests)

BACKGROUND

In vitro assays that measure cell viability and proliferation are useful for the quantitation of a cell population's response to exogenous factors and compounds. Cell proliferation assays have utilized the uptake of radiolabeled thymidine into cellular DNA, however, this method is time consuming and involves the use of hazardous materials. An alternative method is provided by the reduction of tetrazolium salts which is widely accepted as a reliable method for examining cell proliferation. The yellow tetrazolium salt 3-[4,5-di-methylthiazol-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 spectroscopy.

INTENDED USE

The TACS® MTT Cell Proliferation Assay allows for the measurement of cell proliferation rates and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. The TACS MTT Cell Proliferation Assay 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 2-8 °C. 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

Do not use past kit expiration date.

Note: *The components for this kit require different storage/shipping temperatures and will arrive in separate packaging.*

PART	PART #	CATALOG # 4890-025-K	CATALOG # 4890-050-K	AMOUNT PROVIDED	STORAGE OF UNOPENED MATERIAL	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
MTT Reagent	4890-25-01	1 vial	2 vials	25 mL/vial	Store at 2-8 °C **in the dark.**	Store at 2-8 °C **in the dark**.
Detergent Reagent	4890-25-02	1 vial	2 vials	250 mL/vial	Store at room temperature.	Store at room temperature.

OTHER MATERIALS & SUPPLIES REQUIRED

- Microplate plate reader: 650 and 570 nm filters
- Inverted microscope
- Multichannel pipette
- Pipette aid
- 37 °C incubator
- 96-well tissue culture (flat bottomed)
- Sterile tubes (5.0 mL)
- Serological pipettes
- Sterile pipette tips

PRECAUTIONS

The acute and chronic effects of overexposure to reagents of this kit are unknown. Safe laboratory procedures should be followed and protective clothing should be worn when handling kit reagents.

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REAGENT PREPARATION

MTT Reagent - The MTT Reagent is supplied ready for use. The MTT Reagent is stable at 2-8 °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 2-8 °C **in the dark**. If the MTT Reagent is blue-green in color do not use and refer to the Data Interpretation and Troubleshooting Guide.

Detergent Reagent - The Detergent Reagent is supplied ready for use. If the Detergent Reagent has been stored at 2-8 °C, warm the bottle for 5 minutes at 37 °C then invert gently to mix and avoid frothing.

ASSAY PROCEDURE

Cells are cultured in 100 µL of culture medium in a 96-well flat-bottomed tissue culture plate. 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/well) and the plate is incubated for 2-12 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.

1. Harvest cells by centrifugation.
   Note: *Adherent cells should be released from their substrate by trypsinization or scraping prior to centrifugation.*
2. Resuspend cells at 1 x 10^6 cells/mL.
3. Prepare dilutions of cells from 1 x 10^4 -1 x 10^6 cells/mL. The number of cells per well required for optimal results will vary depending upon cell type, culture conditions, etc.
4. In triplicate, pipette 100 µL/well of each dilution, resulting in 10^3-10^5 cells/well. Include three cell culture medium-only controls. Wells containing only medium provide the blank for the absorbance readings.
5. Incubate the cells for 2-24 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.
6. Add 10 µL of MTT Reagent to each well. If more than 100 µL of medium was used per well increase the amount of MTT Reagent used accordingly (i.e. for 250 µL of medium use 25 µL of MTT Reagent).
7. Return plate to the cell culture incubator for 2-4 hours until purple dye is visible. Periodically view the cells under an inverted microscope for the formation of intracellular punctate purple precipitate. Longer periods of incubation for up to 24 hours may be required for some cell types.
8. When the purple precipitate is clearly visible under the microscope, add 100 µL of Detergent Reagent to all wells. Do not shake.
9. Leave plate with cover in the dark for 2-4 hours or overnight at room temperature. Samples can be read as early as 2 hours after adding Detergent Reagent. 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 °C but room temperature is usually adequate.
10. 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 blank wells should give values close to zero (+/- 0.1).
11. 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.
12. Using the cell number determined in Step 11, repeat TACS MTT Cell Proliferation Assay (Steps 4–10) with your experimental samples.

CONTROLS

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 0.75-1.25. The cell number for plating should be determined using the procedure described in the Assay Procedure.

DATA INTERPRETATION & 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 2-8 °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 density per well is too low.	Increase cell density at plating.
	Incubation time for reduction of MTT is 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 is 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.
Absorbance readings too high	Cell number per well is too high.	Decrease cell density at plating.
	Contamination of culture with bacteria, yeast.	Discard. View wells prior to addition of MTT Reagent to check for contamination.
Replicates have different values	Inaccurate plating or pipetting.	Increase accuracy of cell plating, check accuracy of pipette.

REFERENCES

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2. Alley, M.C. *et al*. (1988) Cancer Res. **48**:589.
3. Mosmann, T. (1983) J. Immunol. Methods **65**:55.