DNAbiotech
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MTT Cell Viability Assay Kit

Catalog no.: DMA100, DMA300, DMA500

DNAbiotech Co.

Intended for research use only

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Introduction

The in vitro determination of toxic effects of u known compounds have been performed by counting viable cells after staining with a vital dye.

Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases.

The MTT method is simple, accurate and yields reproducible results. The key component is (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) or MTT. Solutions of MTT, dissolved in medium or balanced salt solutions without phenol red, are yellowish in color. Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, yielding purple formazan crystals which are insoluble in aqueous solutions. The crystals are dissolved in acidified isopropanol.

The resulting purple solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material.

Applications

- Cell Proliferation: effects of cytokines, growth factor, nutrients.
- Cytotoxicity and Apoptosis: evaluation of toxic compounds, anti-cancer antibodies, toxins, environmental pollutants etc.
- Drug Discovery: high-throughput screen for toxic and anticancer drugs.

Storage Conditions and Reagent Preparation:

Kit will arrive packaged as a 4° C & will performed as specified if stored as directed and used before expiration date.

Materials Supplied

No.	Item	QTY	Storage
1*	MTT Reagent	5 mgr/Vila	4° C
2	Sterilized PBS	10 mL	4° C
3**	Detergent Reagent	55 mL	RT
4	Ready to use PBS powder	For 500 mL	RT

*DMA100: 1 vial, DMA300: 3 vials and DMA500: 5 vials

** DMA100: 15, DMA300: 35 vials and DMA500: 55 mL

If any of the items listed above are missing or damaged, please contact us at www.dnabiotech.ir.

User Supplied Reagents and Equipment

- 96-well clear plate with flat bottom.
- Multi-well spectrophotometer (ELISA reader).

Reagent preparation

Add 1 mL of sterilized PBS to each vial of MTT reagent by vortxing. In the event that the powder doses not completely dissolve, the undissloved materials can be removed by filtration or centrifugation. Each vial of **reconstitutd MTT** provides the possibility of 100 reactions. If the entire vial of the MTT reagent will not be used in a single experiment, it is recommended that you aliquot it into smaller sizes and store at -20° C. by this way, MTT reagent will be stable for several months. Avoid repeated frees/thaw.

Procedure

This method of monitoring in vitro cytotoxicity is well suited for use with multiwell plates. For best results, cells in the log phase of growth should be employed and final cell number should not exceed 10^6 cells/cm2. Each test should include a blank containing complete medium without cells.

NOTE1: Cells seeded at densities between 5,000-10,000 cells/well should reach optimal population densities within 48-72 hours. We recommend using appropriate incubation time depending on the individual cell type and cell concentration used.

NOTE2: Bacteria, mycoplasma and other microbial contaminants may also

cleave the MTT tetrazolium ring. Cultures containing microorganisms should not be assayed using this method.

1. Grow cells at varying densities (10^6 cells per ml) in a clear plate according to the desired protocol. Dissolve compounds of interest in an appropriate solvent. Treat cells with compound for desired time period.
2. Remove cultures from incubator into laminar flow hood or other sterile work area.
3. do one of the following methods based on your research:
   a) Add **reconstituted MTT** in an amount equal to 10% of the culture medium volume (usually 100 ul).
   b) For adherent cells, carefully discard the media. For suspension cells, spin the 96-well plate at 1,000 X g, 4°C for 5 min. in a microplate compatible centrifuge and carefully discard the media. Add 100 ul MTT working solution (usually 10 ul of **reconstituted MTT** and 90 ul of sterilizd PBS)
4. Return cultures to incubator for 3-4 hours depending on cell type and maximum cell density. (An incubation period of 2 hours is generally adequate but may be lengthened for low cell densities or cells with lower metabolic activity.) Incubation times should be consistent when making comparisons.
5. After the incubation period, remove cultures from incubator and dissolve the resulting formazan crystals by adding an amount of **Detergent reagent** equal to the original culture medium volume (usually 100 ul).
6. Gentle mixing in a shaker will enhance dissolution. Occasionally, especially in dense cultures, pipetting up and down may be required to completely dissolve the MTT formazan crystals.
7. Spectrophotometrically measure absorbance at a wavelength of 570 nm. Measure the background absorbance of multiwell plates at 690 nm and subtract from the 570 nm measurement.

- Tests performed in multiwell plates can be read using the appropriate type of plate reader or the contents of individual wells may be transferred to appropriate size cuvets for spectrophotometric measurement.

Data analysis cell proliferation assays

- Average the duplicate reading for each sample.
- Subtract the culture medium background from your assay reading. This is the corrected absorbance.
- Amount of absorbance is proportional to cell number.

NOTE: for cell counting, a standard curve can be established with known cell number and fixed incubation times with the assay reagent.

Cell cytotoxicity assays

- Average the duplicate reading for each sample.
- Subtract the culture medium background from your assay readings. This is the corrected absorbance.
- Calculate percentage cytotoxicity with the following equation, using corrected absorbance:

$$\%\ Cytotoxicity = (100\ x\ (Control - Sample))\ /\ Control$$

Typical Data

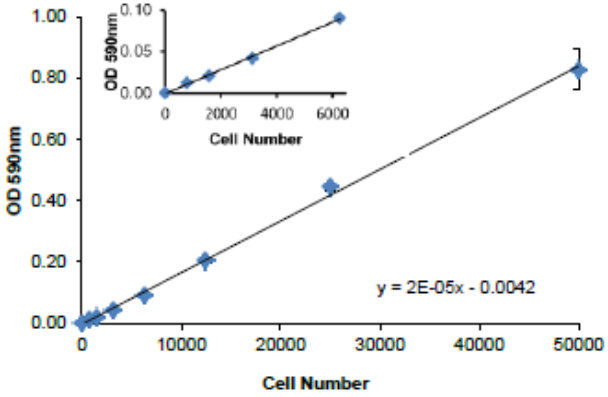

Figure: MTT Cell Proliferation Assay: HeLa cells were grown in DMEM media supplemented with 10% FBS, harvested using trypsin and counted using Trypan blue and a hemocytometer. Cells were serially diluted in a clear cell culture plate and incubated for 3 hrs with MTT Reagent at 37°C. After incubation, cells were treated with MTT Solvent for 15 min. at room temperature. Absorbance was measured at 590 nm. Inset graph is an expanded segment of the assay data at lower cell number per well.

Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold reagent	Warm reagent to assay temperature
Assay not working	Plate read at incorrect wavelength	Check equipment and filter settings of instrument
Unanticipated result	Measured at incorrect wavelength	Check equipment and filter settings of instrument

References

1. J Immunol Methods 65, 55 (1983); 2. J Neurochem 69, 581 (1997); 3. Arch Biochem Biophys 303, 474 (1993); 4. Cancer Res 51, 2515 (1991); 5. J Immunol Methods 168, 253 (1994); 6. Cancer Res 47, 943 (1987); 7. Br J Cancer 56, 279 (1987); 8. J Immunol Methods 93, 157 (1986); 9. J Immunol Methods 130, 149 (1990).
2. Mossman, T. [1983] Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65:55-63.
3. Takeuchi, H. et al. [1991] An application of tetrazolium (MTT) colorimetric assay for the screening of anti-herpes simplex virus compounds. J. Virol. Methods 33:61-71.