IN VITRO TOXICOLOGY ASSAY KIT
MTT BASED
Stock No. TOX-1
Store at 2-8 °C

This kit is designed for determining cell number spectrophotometrically as a function of mitochondrial activity in living cells.

IT IS RECOMMENDED THAT THE ENTIRE PROTOCOL BE REVIEWED BEFORE STARTING THE ASSAY.

PRODUCT DESCRIPTION
Traditionally, the in vitro determination of toxic effects of unknown 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.

REAGENT
For Research Use Only.
Not for Use in Diagnostic Procedures.

KIT COMPONENTS

<table>
<thead>
<tr>
<th>Catalog No.</th>
<th>Item</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>M-5655</td>
<td>MTT</td>
<td>5</td>
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<td></td>
<td>15 mg/vial in serum vial</td>
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<tr>
<td>M-8910</td>
<td>MTT Solubilization Solution</td>
<td>1</td>
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<tr>
<td></td>
<td>10% Triton X-100 plus</td>
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<td></td>
<td>0.1 N HCl in anhydrous isopropanol, 125 ml</td>
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WARNING: Components in this kit should be carefully handled when using. MTT may be harmful if swallowed, inhaled or absorbed through skin. MTT may alter genetic material. MTT SOLVENT is flammable and corrosive.

PRODUCT STORAGE
Kit components should be stored at 2-8 °C.

PROCEDURE
The MTT 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/cm$^2$. Each test should include a blank containing complete medium without cells.

NOTE: Bacteria, mycoplasma and other microbial contaminants may also cleave the MTT tetrazolium ring. Cultures containing microorganisms should not be assayed using this method.

1. Remove cultures from incubator into laminar flow hood or other sterile work area.
2. Reconstitute each vial of MTT [M-5655] to be used with 3 ml of medium or balanced salt solution without phenol red and serum. Add reconstituted MTT in an amount equal to 10% of the culture medium volume.
3. Return cultures to incubator for 2-4 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.

4. After the incubation period, remove cultures from incubator and dissolve the resulting formazan crystals by adding an amount of MTT Solubilization Solution [M-8910] equal to the original culture medium volume.

5. Gentle mixing in a gyratory shaker will enhance dissolution. Occasionally, especially in dense cultures, pipetting up and down [trituration] may be required to completely dissolve the MTT formazan crystals.

6. 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.

POSSIBLE SOURCES OF ERROR

1. Reconstituted MTT solution is stable for at least 6 months when stored frozen (-0 °C). Storage of reconstituted MTT solution at 2-8 °C for more than 2 weeks may cause decomposition and yield erroneous results.

2. Microbial contamination will contribute to the cleavage of MTT and the formation of MTT formazan, yielding erroneous results.

3. Uneven evaporation of culture fluid in wells of multiwell plates may cause erroneous results.

4. High protein levels may form a precipitate when MTT Solubilization Solution is added. Samples with protein concentrations equivalent to 10% fetal bovine serum (4 mg protein/ml of sample) seem acceptable. Sera or serum products with higher protein concentrations may need to be used at lower percentages.

5. Media and salt solutions with phenol red can be used but will contribute to higher background absorbance and can decrease sensitivity.

REFERENCES


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Stock No. TOX-1
7H258