



3050 Spruce Street  
Saint Louis, Missouri 63103 USA  
Telephone 800-325-5832 • (314) 771-5765  
Fax (314) 286-7828  
email: techserv@sial.com  
sigma-aldrich.com

## Product Information

### 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

<u>Catalog No.</u>	<u>Item</u>	<u>Quantity</u>
M-5655	MTT 15 mg/vial in serum vial	5
M-8910	MTT Solubilization Solution 10% Triton X-100 plus 0.1 N HCl in anhydrous isopropanol, 125 ml	1

**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<sup>2</sup>. 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 cuvetts 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

1. Mossman, T. [1983] Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 65:55-63.
2. Denizot, F. and Lang, R. [1986] Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J. Immunol. Methods* 89:271.
3. Carmicheal, J. et al. [1987] Evaluation of a tetrazolium-based, semi-automated colorimetric assay: assessment of chemosensitivity testing. *Cancer Research* 47:936-942.
4. Edmondson, J. et al. [1988] A rapid and simple MTT-based spectrophotometric assay for determining drug sensitivity in monolayer cultures. *J. Tissue Cult. Methods* 11:15-17.
5. Vistica, D. et al. [1991] Tetrazolium-based assays for cellular viability: a critical examination of selected parameters affecting formazan production. *Cancer Research* 51:2515-2520.
6. 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.

IN VITRO TOXICOLOGY ASSAY KIT  
 MTT BASED  
 Stock No. TOX-1  
 7H258

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.