



Product Information

MTT

Product No. M 5655

(3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Thiazolyl blue)

APPLICATIONS

MTT is a water soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by dehydrogenase enzymes (1). This water insoluble formazan can be solubilized using isopropanol or other solvents and the dissolved material is measured spectrophotometrically yielding absorbance as a function of concentration of converted dye.

The cleavage and conversion of the soluble yellow dye to the insoluble purple formazan has been used to develop an assay system alternative to the conventional ³H-thymidine uptake and other assays for measurement of cell proliferation. Active mitochondrial dehydrogenases of living cells will cause this conversion. Dead cells do not cause this change. This has been applied in measurement of interleukin-2 activity in a multiwell assay (2). Modification has improved the sensitivity (3). Other uses such as measurement of cytotoxicity (4) and cell number have also been developed.

In our testing we dissolve MTT, (Product No. M 5655), 5 mg/ml in RPMI-1640 without phenol red. This is available as a powder (Product No. R 8755) or liquid (Product Nos. R 8632 or R 7509). The solution is filtered through a

0.2 µm filter and stored at 2-8°C for frequent use or frozen for extended periods.

Routinely, MTT stock solution (5 mg/ml) is added to each culture being assayed to equal one tenth the original culture volume and incubated for 3 to 4 hr. At the end of the incubation period the medium can be removed if working with attached cells and the converted dye may be solubilized with acidic isopropanol (0.04-0.1 N HCl in absolute isopropanol). When working with suspension cells the dye is added directly and dissolution is accomplished by trituration. Absorbance of converted dye is measured at a wavelength of 570 nm with background subtraction at 630-690 nm.

MTT may also be used to score hybridoma development or clonal development. Clones will convert the dye and become readily visible without magnification. It should be noted that MTT is a mutagen and the resultant cells may be affected. The concentration used may be reduced by dilution of the stock solution (5 mg/ml) to 0.1 mg/ml and adding a tenth volume to each well. Incubation should be monitored by observing for stained clones. Cells can be recovered by gently washing the cells and adding growth medium.

SUMMARY

MTT stock solution	5 mg/ml
Typical use	Add 1/10th of culture volume
Solvent	0.04-0.1 N HCl in isopropanol
Spectrophotometric reading	570 nm
Background wavelength	630-690 nm

1. Slater T.F. et al. (1963). *Biochim. Biophys. Acta* 77: 383.
2. Mossman, T. (1983). *J. Immunol. Methods* 65: 55.
3. Denizot, F. and Lang, R. (1986). *J. Immunol. Methods* 89: 271.
4. Carmichael, J. et al. (1987). *Cancer Research* 47: 936