

Product Information

IN VITRO TOXICOLOGY ASSAY KIT XTT BASED

Product Number **TOX-2**

Storage Temperature -0°C

Product Description

This kit provides a means 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.

Traditionally, the in vitro determination of toxic effects of unknown compounds has 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 XTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases.

The XTT method is simple, accurate and yields reproducible results. The key component is the sodium salt of (2,3-bis[2-Methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide inner salt) or XTT. Solutions of XTT, prepared in medium or balanced salt solutions without phenol red, are yellowish in color. Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring of XTT yielding orange formazan crystals which are soluble in aqueous solutions. The resulting orange solution is spectrophotometrically measured. The bioreduction of XTT is inefficient but can be potentiated by the addition of an electron coupling agent such as phenazine methosulfate (PMS) to the reaction. An increase or decrease in cell numbers results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material.

Components

X-4751 XTT + 1% PMS, 5 mg in
 5 mg/vial serum vial

Precautions and Disclaimer

For Research Use Only.

Not for Use in Diagnostic Procedures.

WARNING: Components in this kit should be carefully handled when using. XTT and PMS may be harmful if swallowed, inhaled or absorbed through skin. XTT and PMS may alter genetic material.

Preparation Instructions

The XTT 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.

NOTE: Bacteria, mycoplasma and other microbial contaminants may also cleave the XTT 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 XTT to be used with 5 ml of medium without phenol red and serum [Note: see below]. Add reconstituted XTT in an amount equal to 20% 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. Gentle mixing in a gyratory shaker will enhance dispersion. Occasionally, especially in dense cultures, pipetting up and down [trituration] may be required to completely disperse the XTT formazan.
5. Spectrophotometrically measure absorbance at a wavelength of 450 nm. Tests performed in multiwell plates can be read using an appropriate type of plate reader. When multiwell plates are used, it is recommended that the absorbance at a reference wavelength of 690 nm be measured and subtracted from the 450 nm measurement. The contents of individual wells may be transferred to appropriate size cuvetts for spectrophotometric measurement.

Storage/Stability

XTT powder and reconstituted XTT solution are stable when stored frozen (-0 °C). 1 mg/ml XTT is a saturated solution and incomplete solubility may appear upon reconstitution, thawing, and/or prolonged usage at room temperature. Solubility of the dye can be enhanced by warming the solution in a 56 °C waterbath.

Specificity**POSSIBLE SOURCES OF ERROR**

1. XTT powder and reconstituted XTT solution are stable when stored frozen (-0 °C). Storage at 4 °C may result in decomposition and yield erroneous results.
2. Microbial contamination will contribute to the cleavage of XTT and formation of XTT formazan yielding erroneous results.
3. Uneven evaporation of culture fluid in wells of multiwell plates may cause erroneous results.
4. Media and salt solutions with phenol red can be used but will contribute to higher background absorbance and can decrease sensitivity.

References

1. Scudiero, D. et al. [1988] Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other cell lines. *Cancer Research* 48:4827-4833.
2. Weislow, O. et al. [1989] New soluble-formazan assay for HIV-1 cytopathic effects: Application to high-flux screening of synthetic and natural products for AIDS-antiviral activity. *J. Natl. Cancer Inst.* 81:577-586.
3. Roehm, N. et al. [1991] An improved colorimetric assay for cell proliferation and viability utilizing the tetrazolium salt XTT. *J. Immunol. Methods* 142:257-265.

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