

Product Information

***In Vitro* Toxicology Assay Kit, XTT based**

Catalog Number **TOX2**Storage Temperature -20°C

TECHNICAL BULLETIN

Product Description

This kit provides a spectrophotometric method for estimating cell number based on the mitochondrial activity in living cells. Traditionally, the toxic effects of unknown compounds have been determined *in vitro* by counting viable cells after staining with a vital dye. Alternative methods include determination of radioisotope incorporation to measure DNA synthesis, counting by automated counters, and other methods, which rely on dyes and cellular activity. This XTT based kit measures the mitochondrial dehydrogenase activity of living cells.

The XTT method is simple, accurate, and yields reproducible results. The key component is the sodium salt of XTT (2,3-bis[2-Methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide inner salt). Solutions of XTT, prepared in medium or a balanced salt solution without phenol red, are yellowish in color. The mitochondrial dehydrogenases of viable cells reduce the tetrazolium ring of XTT, yielding an orange formazan derivative, which is water soluble. The absorbance of the resulting orange solution is measured spectrophotometrically. 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 viable cells relative to control cells, results in an accompanying change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material.

Reagent

Each kit is sufficient for 1,000 tests

XTT with 1% PMS 5 × 5 mg
packaged in serum vials
(Catalog Number X4751)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

It is recommended to review the entire bulletin before starting the procedure.

Preparation Instructions

Prepare a XTT Stock Solution by reconstituting a vial of the XTT with 1% PMS mixture (Catalog Number X4751) with 5 ml of medium or a balanced salt solution without phenol red or serum. At a concentration of 1 mg/ml, XTT forms a saturated solution and incomplete solubility may be observed upon reconstitution, thawing, and/or prolonged usage at room temperature. Warming the solution in a 56°C water bath will help dissolve the dye.

Note: Media and salt solutions with phenol red can be used, but will contribute to a higher background absorbance, which may decrease sensitivity.

Storage/Stability

It is recommended to store the XTT with 1% PMS mixture (Catalog Number X4751) and the XTT Stock Solution at -20°C . Storage at $2-8^{\circ}\text{C}$ may result in decomposition and yield erroneous results.

Procedure

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 procedure should include a blank containing complete medium without cells.

Note: Bacteria, mycoplasma, and other microbial contaminants will also contribute to the reduction of the XTT tetrazolium ring and formation of XTT formazan, yielding erroneous results. Cultures containing microorganisms should not be assayed using this procedure.

1. Remove cultures from incubator into laminar flow hood or other sterile work area.
2. Add a volume of reconstituted XTT Stock Solution equal to 20% of the culture medium volume to be tested.
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 for similar samples should be consistent to make comparisons.
Notes: Gentle mixing on a gyratory rocker will enhance dispersion of the XTT Stock Solution. Occasionally, especially in dense cultures, pipetting up and down may be required to completely disperse the XTT formazan.

Uneven evaporation of the fluid in the wells of plates may cause erroneous results.

4. Measure the absorbance at 450 nm. Tests performed in multiwell plates can be read with an appropriate plate reader. Alternatively, the contents of an individual well may also be transferred to an appropriate cuvette for spectrophotometric measurement.
Note: When multiwell plates are read with an appropriate plate reader, it is recommended to also measure the absorbance at 690 nm. Use the absorbance at 690 nm as a background measurement and subtract it from the 450 nm value.

References

1. Scudiero, D. et al., 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 (1988).
2. Weislow, O. et al., 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 (1989).
3. Roehm, N. et al., An improved colorimetric assay for cell proliferation and viability utilizing the tetrazolium salt XTT. *J. Immunol. Methods*, **142**, 257-265 (1991).

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