

3.2.2 Assays that measure metabolic activity

Living (metabolically active) cells reduce tetrazolium salts to colored formazan compounds; dead cells do not. Thus, tetrazolium salt-based colorimetric assays detect viable cells exclusively. Because they are sensitive, these assays can readily be performed in a microplate with relatively few cells.

Since a cytotoxic factor will reduce the rate of tetrazolium salt cleavage by a population of cells, these metabolic activity assays are frequently used to measure factor-induced cytotoxicity or cell necrosis^{53, 54}. Applications include:

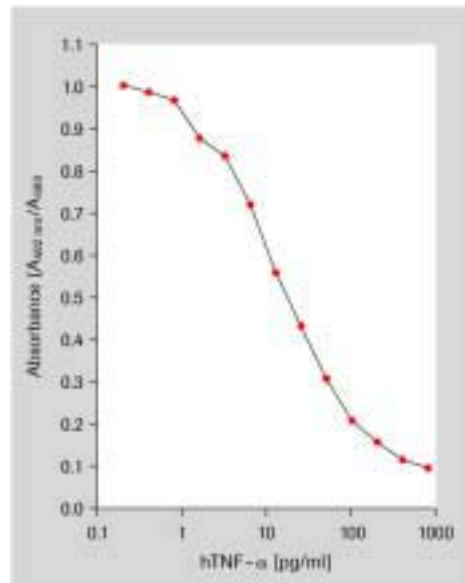
- Assessment of growth-inhibitory or cytotoxic effects of physiological mediators (Figure 49).
- Analysis of the cytotoxic and cytostatic effects of potential anti-cancer and other drugs (Figure 50).
- Analysis of cytopathic effects of viruses and screening of compounds with potential anti-viral activity.
- Screening of antibodies for growth-inhibiting potential.

Roche Applied Science offers three microplate-based metabolic activity assays. All may be used to assay factor-induced cytotoxicity or necrosis. They are:

- **Cell Proliferation Kit I (MTT)**, Cat. No. 11 465 007 001, in which metabolically active cells cleave the tetrazolium salt MTT to a water-insoluble formazan that can be solubilized and quantitated with an ELISA plate reader (for a more detailed description of this kit, see page 85 in this guide).
- **Cell Proliferation Kit II (XTT)**, Cat. No. 11 465 015 001, in which metabolically active cells cleave the modified tetrazolium salt XTT to a water-soluble formazan, which may be directly quantitated with an ELISA plate reader (for a more detailed description of this kit, see page 86 in this guide).
- **Cell Proliferation Reagent WST-1**, Cat. No. 11 644 807 001, a modified tetrazolium salt that can be cleaved by metabolically active cells to a water-soluble formazan, which may be directly quantitated with an ELISA plate reader (for a more detailed description of this reagent, see page 87 in this guide).

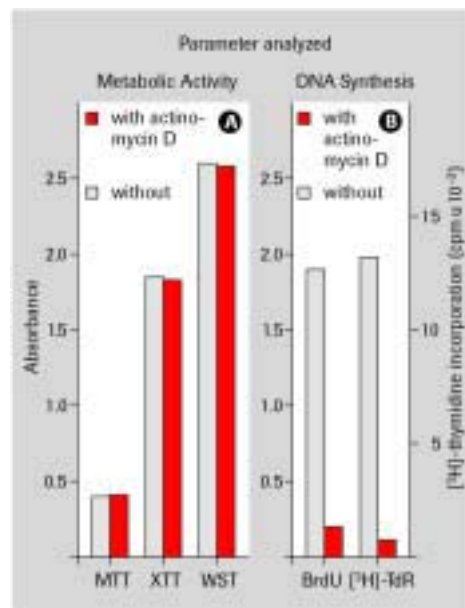
Note: Since proliferating cells are metabolically more active than non-proliferating (resting) cells, these tetrazolium salt-based assays are also frequently used to measure cell activation and proliferation. For a full discussion of this application, see Section B 2.1.1 on page 82 of this guide.

For a more complete discussion of the principles behind these metabolic assays, see the topic, “Biochemical and cellular basis of cell proliferation assays that use tetrazolium salts” (Appendix, page 121) in this guide.



▲ **Figure 49: Measurement of the cytotoxic effects of human Tumor Necrosis Factor alpha (hTNF- α) on the mouse fibrosarcoma cell line WEHI-164.** Cells in culture (10^6 cells/ml) were preincubated with actinomycin C (1 μ g/ml) for 3 h. Aliquots of these pretreated cells were transferred to a microtiter plate (5×10^4 cells/well) and incubated with actinomycin C and various amounts of hTNF-alpha for 24 h. Cellular response to TNF was measured with the Roche Applied Science Cell Proliferation Kit II (XTT) and plotted against TNF concentration.

Result: Under the assay conditions, 50% of the WEHI-164 cells were killed by a TNF concentration of 35–40 μ g/ml.



▲ **Figure 50: Differentiation of cytotoxic and cytostatic effects of actinomycin D.** A549 cells were added to a microtiter plate (10^4 cells/well) and incubated with (■) or without (□) actinomycin D (10 ng/ml) for 20 h.

Graph A: Some aliquots of actinomycin-treated cells were assayed for cytotoxic effects (changes in metabolic activity). These cells were assayed with either the Cell Proliferation Kit I (MTT), Cell Proliferation Kit II (XTT), or Cell Proliferation Reagent WST-1 (WST). Cells were incubated with each tetrazolium salt for 4 h, then analyzed on an ELISA plate reader.

Graph B: Other aliquots of actinomycin-treated cells were assayed for cytostatic effects (suppression of DNA synthesis). These cells were incubated with either non-radioactive bromodeoxyuridine (BrdU) or tritiated thymidine ($[^3\text{H}]\text{-TdR}$). Incorporation of BrdU into DNA was determined with the Cell Proliferation ELISA, BrdU (colorimetric). Incorporation of $[^3\text{H}]\text{-TdR}$ into DNA was determined by liquid scintillation counting.

Result: Although actinomycin D is not significantly cytotoxic (as indicated by graph A) under these conditions, it does have a profound cytostatic (proliferation-inhibiting) effect (as indicated by graph B).