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REAGENT A FROM
COLORIMETRIC (MTT) ASSAY FOR CELL SURVIVAL AND PROLIFERATION

CATALOG NUMBER: CT01-5

LOT NUMBER:

QUANTITY: 5 vials (CT0-A)

DESCRIPTION: **Reagent A:** MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), 50mg/vial. MTT is a pale yellow substrate that is cleaved by living cells to yield a dark blue formazan product. This process requires active mitochondria, and even freshly dead cells do not cleave significant amounts of MTT. Reagent A is a component of Millipore's MTT assay kit, Catalog Number CT01/CT02.

REQUIRED BUT NOT PROVIDED: **Solution B (not provided):** PBS pH 7.4, 60 mL
Solution C (not provided): Color development solution (isopropanol with 0.04 N HCl), 500 mL

REAGENT PREPARATION: For every 1,000 assays to be performed, add 10 mL Solution B to one vial of Reagent A. Mix well, sterile filter and keep in the dark at 4° C until used. **It may take overnight to dissolve. Do not heat solution.** When absolutely required to dissolve crystals, adjust pH with 1-2 drops of HCl. The AB mixture is stable for several weeks under these conditions.

PROCEDURE:

1. Carry out a lymphokine, mitogen, or complement-mediated cytotoxicity assay using standard methods, in 96-well flat-bottomed tissue culture plates of good optical quality (e.g. Falcon). The final volume of tissue culture medium in each well should be 0.1 mL, and the medium (e.g. RPMI or DMEM) may contain up to 10% Fetal Bovine Serum.
2. At the end of the assay add 0.01 mL AB Solution (MTT) to each well. Mix by tapping gently on the side of the tray.
3. Incubate at 37°C for cleavage of MTT to occur. Optimal times may vary according to the assay, but four hours is suitable for most purposes. At the end of this time, the MTT formazan produced in wells containing live cells will appear as black, fuzzy crystals on the bottom of the well.
4. Add 0.1 mL Solution C to each well. Mix thoroughly by repeated pipetting with a multichannel pipettor. The HCl converts the phenol red in tissue culture medium to a yellow color that does not interfere with MTT formazan measurement. The isopropanol dissolves the formazan to give a homogeneous blue solution suitable for absorbance measurement.
5. Within an hour, measure the absorbance on an ELISA plate reader with a test wavelength of 570 nm and a reference wavelength of 630 nm. After several hours at room temperature, serum proteins may begin to precipitate due to the acid/alcohol. Chilling the plates will hasten the precipitation. If the plates must be stored before measuring, keep at 4° C before adding the acid/alcohol, then warm to room temperature and add acid/alcohol just before reading.

RESULTS: The MTT assay will normally detect 200 to 50,000 cells of a typical cell line, although 1,000 to 50,000 is the useful range. This number may vary for other cell types. Cytotoxic assays should be set up so that the control, unlysed cells give a signal of 0.2 to 0.4, and proliferation assays should yield a similar value at plateau concentrations. This corresponds to about 20-50,000 cells per well with a typical cell line.

Absorbance is directly proportional to the number of cells; actual cells do not absorb significantly, even up to concentrations of l x 10^6 cells/mL.

STORAGE: Maintain at 2-8°C for up to six months. The Reagent A / Solution B mixture is stable at 2-8°C

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for up to two weeks.

REFERENCES:

1. Mosmann, T., "Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays", *J. Immunol. Methods*, **65**:55-63 (1983).
2. Green, L.M., *et al*., *J. Immunol. Methods*, **70**:257-268 (1984).

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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