



Product Information

INVITRO TOXICOLOGY ASSAY KIT

Sulforhodamine B Based

Product No. TOX-6

Store at Room Temperature

IT IS RECOMMENDED THAT THE ENTIRE PROTOCOL BE REVIEWED BEFORE STARTING THE ASSAY.

Product Description

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 Sulforhodamine B Assay system is a means of measuring total biomass by staining cellular proteins with the Sulforhodamine B.

The Sulforhodamine B method, as originally developed by Skehan et al.¹, is simple, accurate and yields reproducible results. The key component is the dye, Sulforhodamine B (Acid Red 52). The cells are briefly washed, fixed and stained with the dye. The incorporated dye is then liberated from the cells in a tris base solution. An increase or decrease in the number of cells (total biomass) results in a concomitant change in the amount of dye incorporated by the cells in the culture. This indicates the degree of cytotoxicity caused by the test material.

Reagents

Catalog No.	Item	Quantity
S 2902	Sulforhodamine B Solution, 0.4% in 1% Acetic Acid	100 ml
T 5288	Trichloroacetic Acid, 50% [Sulforhodamine B Assay Fixative Solution]	30 ml
A 8915	Acetic Acid Solution, 10% [Sulforhodamine B Assay Wash Solution, 10X]	100 ml
T 5413	Tris Base Solution, 10 mM [Sulforhodamine B Assay Solubilization Solution]	125 ml

Precautions and Disclaimer

WARNING: Components in this kit should be carefully handled when using. Sulforhodamine B Assay Fixative may be harmful if swallowed, inhaled, or absorbed through skin. This product can cause severe chemical burns.

Reagent

For R&D use only. Not for drug, household or other uses.

Procedure

The Sulforhodamine B method of monitoring in vitro cytotoxicity is well suited for use with multiwell plates. Optimally, cells in the log phase of growth should be used and final cell number should not exceed 10^6 cells/cm². Each test should include a blank containing complete medium without cells.

1. Remove cultures from incubator into laminar flow hood or other sterile work area.
2. Prepare fixative (50% TCA) by adding 22 ml of reagent grade water to the bottle of TCA (T5288). Fix the cells by gently layering $\frac{1}{4}$ volume of cold 50% TCA on top of the growth medium.
3. Incubate plate for 1 hour at 4 °C and then rinse with water several times to remove TCA, serum proteins, etc. Plates are air dried and stored until use. Blank background optical density is measured in wells incubated with growth medium without cells.
4. Add Sulforhodamine B Solution [0.4%] in an amount sufficient to cover the culture surface area (approx. 50% of the culture medium volume).
5. Allow cells to stain for 20-30 minutes.

6. Prepare the wash solution by diluting the 10% acetic acid with 9 parts of water. At the end of the staining period, the stain is removed and the cells rinsed quickly with 1% acetic acid. Repeat until unincorporated dye is removed. Keep wash times to a minimum to reduce desorption of protein-bound dye. After being rinsed, the cultures are air dried until no moisture is visible.
7. The incorporated dye is then solubilized in a volume of Sulforhodamine B Assay Solubilization Solution (10 mM Tris) equal to the original volume of culture medium. Allow cultures to stand for 5 minutes at room temperature. Gentle stirring in a gyratory shaker or pipetting up and down (trituration) will enhance mixing of the dye.
8. Spectrophotometrically measure absorbance at a wavelength of 565 nm. If intense color is observed a suboptimal wavelength (490-530 nm) can be used to facilitate reading of wells. Measure the background absorbance of multiwell plates at 690 nm and subtract from the measurement at 565 nm.
9. Tests performed in multiwell plates can be read using an appropriate type of plate reader or the contents of individual wells may be transferred to appropriate size cuvetts for spectrophotometric measurement.

Results

Possible Sources of Error

1. Sulforhodamine B may precipitate in solution upon storage. If precipitated dye crystals interfere with the assay, the dye solution may be filtered using a syringe filter before adding to the cell cultures.
2. Prolonged exposure of the cells to the wash solution can result in leaching of the dye into the wash solution.
3. Uneven evaporation of culture fluid in wells of multiwell plates may cause erroneous results.

References

1. Skehan, P. et al. [1989] Evaluation of Colorimetric Protein and Biomass Stains for Assaying Drug Effects Upon Human Tumor Cell Lines. Proc. Amer. Assoc. Cancer Res. 30:2436.
2. Skehan, P. et al. [1990] New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. Journal National Cancer Institute 82:1107-1112.

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