3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

***In Vitro* Toxicology Assay Kit**
Sulforhodamine B based

Catalog Number **TOX6**
Store at Room Temperature

TECHNICAL BULLETIN

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, is simple, accurate and yields reproducible results.[1] 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 with 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.

Components

Sulforhodamine B Solution 0.4% in 1% Acetic Acid Catalog Number S2902	100 mL
Trichloroacetic Acid, 50% Sulforhodamine B Assay Fixative Solution Catalog Number T5288	30 mL
Acetic Acid Solution, 10% Sulforhodamine B Assay Wash Solution, 10× Catalog Number A8915	100 mL
Tris Base Solution, 10 mM Sulforhodamine B Assay Solubilization Solution Catalog Number T5413	125 mL

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 that the entire protocol be reviewed before starting the assay.

Preparation Instructions

50% (w/v) TCA Solution – Prepare the fixative solution by adding 22 mL of reagent grade water to the bottle of 15 grams of solid trichloroacetic acid (Catalog Number T5288). This will give 30 mL of a 50% (w/v) TCA Solution.

Wash Solution – Prepare the wash solution by diluting the 10% Acetic Acid Solution (Catalog Number A8915) 10-fold with water (9 volumes of water to 1 volume of 10% Acetic Acid Solution).

Storage/Stability

Store the kit at room temperature.

Procedure

The Sulforhodamine B method of monitoring *in vitro* cytotoxicity is well suited for multiwell plates. Optimally, cells in the log phase of growth should be used and the final cell number should not exceed 1×10^6 cells/cm^2. 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. Fix the cells by gently layering ¼ volume of cold 50% (w/v) TCA Solution 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 solution, 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 0.4% Sulforhodamine B Solution (Catalog Number S2902) in an amount sufficient to cover the culture surface area (~50% of the culture medium volume).

5. Allow cells to stain for 20–30 minutes.

6. At the end of the staining period, the stain is removed and the cells rinsed quickly with Wash Solution (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. Measure the background absorbance of multiwell plates at 690 nm and subtract from the measurement at 565 nm.
   Note: If intense color is observed a suboptimal wavelength (490–530 nm) can be used to facilitate reading of wells.

9. Tests performed in multiwell plates can be read using an appropriate plate reader or the contents of individual wells may be transferred to appropriate size cuvettes 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., Evaluation of Colorimetric Protein and Biomass Stains for Assaying Drug Effects Upon Human Tumor Cell Lines. Proc. Amer. Assoc. Cancer Res., **30**, 2436 (1989).
2. Skehan, P. et al., New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. Journal National Cancer Institute, **82**, 1107-1112 (1990).

DF,MAM 05/12-1