SERVA
Electrophoresis

PRODUCT INFORMATION

Sulforhodamine B Cytotoxicity Assay Cat. No. 39906

Product Description:

General	The assay, which was developed in 1990, remains one of the most widely used methods for in vitro cytotoxicity screening. The assay relies on the ability of SRB to bind to protein components of cells that have been fixed to tissue culture plates. The SRB assay is sensitive, simple, reproducible and more rapid than the formazan-based assays and gives better linearity, a good signal-to-noise ratio and has a stable end-point that does not require a time-sensitive measurement, as do the MTT or XTT assays. This assay has been used for high-through put drug screening at the National Cancer Institute (NCI).
Content	The kit is sufficient for 1000 reactions.
Features	• SRB is a bright-pink aminoxanthene dye with two sulfonic groups that bind to basic amino acid residues under mild acidic conditions, dissociate under basic conditions. As the binding of SRB is stoichiometric, the amount of dye extracted from stained cells is directly proportional to the cell mass. • The fixed dye is solubilized and measured photometrically at OD 540 nm with a reference filter of 690 nm. The OD values correlate with total protein content and therefore with cell number.
Storage	Store all kit components at + 15 °C to 30 °C, protected from light.

Assay Procedure

Wash Solution: Prepare the wash solution by diluting the Dye Wash Solution 10X 10-fold with water (9 volumes of water plus 1 volume of Dye Wash Solution 10X).

Sulforhodamine B Solution: Dissolve content of SRB Dye vial (0.4 g) with 100 ml Wash Solution (Dye Wash Solution 1X). Store the solution in a dark (amber) bottle at room temperature.

Some components of this kit are potentially carcinogenic or corrosive; it is advisable to work in a hood and to wear glasses, gloves and a mask.

1. Cells are grown in a 96-well plate in 200 µl growth medium.
   Note: The optimal conditions for monitoring cytotoxicity are to have the cells in the log phase of growth and not exceed to 10^6 cells/cm^2.
2. Without removing the cell culture, gently add 100 µl cold Fixative Reagent to each well.
3. Incubate the plate for 1 hour at 4 °C.

4. Wash the wells four times with 200 µl/well distilled or de-ionized water and remove excess water using paper towels. The wash removes excess fixative and serum proteins.
5. Incubate in a 37 °C incubator for 45minutes to remove excess wash (or air dry the plates overnight if storage is required).

NOTE: After fixing and drying, the plates can be stored indefinitely at room temperature.

6. Add 100 µl Sulphorhodamine B Solution to each well of the dry 96-well plates and allow staining at room temperature for 30 min in the dark.
7. Remove the Sulphorhodamine B Solution by washing the plates quickly with Wash Solution, four times, to remove unbound dye.
8. Dry the washed plates in the air until no moisture is visible.

NOTE: Once the Sulphorhodamine B Solution has been added the plates should be protected from light.

9. Solubilise the bound SRB by adding 200 µl of SRB Solubilization Buffer to each well and shaking for 5 min on a shaker platform.
10. Read the optical density (OD) at 550 -580nm, 565nm is the absorption maximum, with a micro plate reader. If intense colour is visualized (> 1.8), a suboptimal wavelength (490-530 nm) may be used instead. Measure the background absorbance with a reference filter at 690 nm.

The OD of SRB in each well is directly proportional to the cell number so the OD values can be plotted against concentration and the IC50 determined.

Calculation

Calculate the percent cytotoxicity for a given experimental treatment, by using the average absorbance values from experimental, cell control.

Ensure that absorbances are blanked with the cell free medium controls.

% Cytotoxicity= (100 x (Cell Control – Experimental)) ÷ (Cell Control)

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SERVA Electrophoresis GmbH • D-69115 Heidelberg • Carl-Benz-Str. 7
Tel.: +49(0)6221 / 138 40-0 • Fax• +49(0)6221 / 138 40-10 • E-Mail:
info@serva.de http://www.serva.de