Canvax Reagents, S.L.U.
Luis de Mercado Street, 19
Boecillo Technological Park
47151, Boecillo
Valladolid, Spain.

Tlf: +34 983 54 85 63
info@canvaxbiotech.com

www.canvaxbiotech.com

DATA SHEET

Version: 04
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1. Identification

Product name

SRB Cytotoxicity Assay

1000 assays

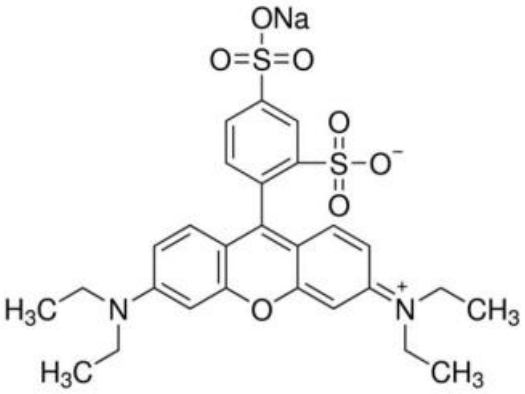
Cat. No: **CA050**

2. Description

The Sulforhodamine B (SRB) cytotoxicity assay is a colorimetric method for assessing cell viability, proliferation and cytotoxicity. The assay is based on the binding of SRB dye to cellular proteins in fixed cells under mildly acidic conditions.

The amount of bound dye is proportional to total cellular protein content and therefore correlates with cell mass and cell number. After staining, unbound dye is removed and the protein-bound SRB is solubilized under basic conditions.

Absorbance is measured at 540 nm, using 690 nm as a reference wavelength for background correction. The assay provides good linearity, a high signal-to-noise ratio and a stable endpoint, making it suitable for routine cytotoxicity, viability and proliferation studies as well as high-throughput screening.



3. Composition

Item	Quantity
SRB Dye (Sulforhodamine B)	0.4 g
Fixative Reagent	2 x 50 mL
Dye Wash Solution 10X	2 x 50 mL
SRB Solubilization Buffer	2 x 100 mL

! Caution

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.

4. Storage specifications

Store all kit components a room temperature, protected from light.

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5. Applications

- Detection of cell toxicity, death, viability or proliferation.
- High throughput screening.

6. Product Feature

- Sensitive colorimetric detection of changes in cell mass.
- Simple and straightforward workflow.
- Reproducible performance.
- Stable endpoint that does not require immediate measurement.
- Suitable for high-throughput screening.
- Non-radioactive.

7. Reagent Preparation

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

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

8. Assay Procedure

1. Cells are grown in a 96-well plate in 200 µl growth medium.
   Note: For optimal performance, cells should be in the logarithmic growth phase and should not exceed a density of 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 45 minutes 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. Solubilize the bound SRB by adding **200 µl of SRB Solubilization Buffer** to each well and shaking for 5 min on a shaker platform.

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10. Measure absorbance with a microplate reader within the 540–580 nm range. The absorption maximum is approximately 565 nm. For standardized measurements, 540 nm is recommended. 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

    The OD of SRB in each well is proportional to cellular protein content and correlates with cell number

11. Calculation of Results:
    Calculate the percentage of cytotoxicity using the average absorbance values of the experimental treatment and the cell control. Absorbance values should be blank-corrected using the cell-free medium controls.

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

7. Further information

Product Use Limitations	This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
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