# Sulforhodamine B (SRB) Cell Cytotoxicity Colorimetric Assay (1000 assays)

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CAT No. ZX-44119-1000

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Application:

Detection of cell viability, cytotoxicity and cell proliferation

**!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!**

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Please read this insert completely prior to using the product.


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## Background

The sulforhodamine B (SRB) cytotoxicity assay is a simple, sensitive and reproducible method for cell density determination, which relies on the binding ability of SRB dye to basic amino-acid residues of cellular proteins. Under mild acidic conditions, the protein components of the cells can bind to SRB dye and are subsequently extracted under basic conditions. The method was developed in 1990, and is still one of the most widely used assays for *in vitro* cytotoxicity screening. As the binding of SRB is stoichiometric, the amount of extracted dye is proportional to cell mass and therefore is indicative of the number of cells in a sample.

The fixed dye is solubilized and colorimetrically measured at OD 565 nm (550-580 nm is acceptable) with a reference filter of 690 nm. The OD values correlate with total protein content and thus with cell number.

In comparison to formazan-based assays (e.g. MTT or XTT assays), SRB provides a higher signal-to-noise ratio and a better linearity with cell number. Additionally, due to its nondestructive and stable endpoint, SRB measurement is not time sensitive. These advantages make the SRB assay suitable for large scale cytotoxicity screening.

## Intended use

Determination of cellular cytotoxicity and cell lysis in response to drug products, toxic compounds, toxins, detergents, environmental pollutants and physical treatment.

## Materials supplied in the Kit

| ***Component*** | ***Quantity*** |
|---|---|
| **Sulforhodamine B (SRB) Dye** | 400 mg |
| **Solubilization Solution** | 200 mL |
| **Wash Solution Conc. (10X)** | 100 mL |
| **Fixation Solution** | 100 mL |

## Storage instruction

All components should be stored at room temperature until the expiration date of the kit and protected from light.

## Materials required but not supplied

Precision pipettes and disposable filter pipette tips

Sterile clear 96 well plate

## Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

## General remarks

- Equilibrate all kit components to room temperature (RT) before use.
- The instruction must be strictly followed.
- Pipette tips should not be used more than once to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

## Reagent preparation

i. **Wash Solution:** Prepare a 1:10 dilution of Wash Solution Concentrate with di$H_2O$ (1 part Wash Solution Conc. with 9 parts di$H_2O$) and mix well.

ii. **SRB Dye Solution:** Dissolve 400 mg of Sulforhodamine B (SRB) Dye in 100 mL of wash solution (prepared in previous step) and mix well. **SRB Dye Solution** must be kept at **Dark.** (we recommend to use dark amber)

## Sample preparation

i. At an appropriate time (72 hours) before the experiment, cultivate cells (5,000–20,000 cells/well) in a 96-well flat-bottom plate containing 200 µL/well of cell culture medium. The appropriate time of incubation may vary based on the cell types and the desired chemical or physical treatments, and must be evaluated by the end user.

ii. Add test compounds and solvent in triplicate wells and incubate the cells in a $CO_2$ incubator at 37°C for an appropriate time.

iii. Each plate must include at least three wells as **Untreated Cells Control** which should be treated with the same solvent but without the potential cytotoxic component; and three wells as **Blank Control** that contain culture medium-only.

- We strongly recommend to determine the optimal cell number and incubation time for your specific cells before performing a large number of SRB assays.

## Assay Procedure

1. Without removing the culture medium, add 100 µL of Fixation Solution to each well.
2. Incubate the plate for 1 hour at 4 °C.

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3. Remove the solution and wash the wells 4 times with $dH_2O$ or $diH_2O$.
4. Remove water using pipettes and tap on paper towels to remove all solution.
5. Incubate at 37°C for 45 minutes (or air-dry overnight) to remove excess water.

- At this stage: The plate can be stored up to 1 month at room temperature for later analysis.

6. Add 100 µL SRB Dye Solution to each well and allow staining at room temperature for 30 min protected from light.

- Due to light sensitivity, after the addition of SRB dye, all steps must be conducted protected from light.

7. Wash the plates quickly with 200 µL Wash Solution 4 times to remove unbound SRB dye.
8. Remove Wash Solution using pipettes and allow the plate to air-dry until all Wash Solution is evaporated.
9. Add 200 µL of Solubilization Solution to each well and shake for 5-10 minutes at RT using a microplate shaker.
10. Read the absorbance signal at 565 nm (550-580 nm is acceptable). Read background absorbance at 690 nm.

- If intense color is observed (OD > 3-3.5), a suboptimal wavelength (490-530 nm) may be used to lower the readings back to the linear range of your instrument.

## Calculation

- Average the triplicate optical density (OD) readings for each standard and sample.
- Subtract the mean ODs of blank (containing only culture medium) from all OD values.
- Calculate the percentage of cytotoxicity as follows:

$$\%\ \boldsymbol{Relative\ Cytotoxicity} = \mathbf{100} \times \frac{\boldsymbol{OD\ Untreated\ Cells\ Control - OD\ \ experimental\ sample}}{\boldsymbol{OD\ Untreated\ Cells\ Control}}$$



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