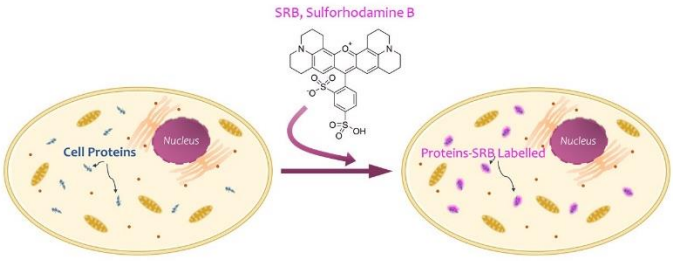
SRB Cytotoxicity Assay Kit

Ordering info

TBK0518. 1,000 assays

Description

SRB Cytotoxicity Assay Kit is an excellent and efficient assay to evaluate cytotoxicity and cell viability. It is based in the staining of cellular proteins with the bright pink aminoxanthane dye, sulforhodamine B (SRB). SRB forms an electrostatic complex with basic amino acid residues in labeling acidic conditions, but it can dissociate under solubilization basic conditions.



The binding of SRB is stochiometric. The incorporated dye solubilized is directly proportional to the cell number. The assay readout is colorimetric at 540 nm with a reference at 690 nm.

Features

- Accurate, simple and reproducible assay.
- Highly sensitive, 1-200% of cell confluence is in a linear range with cell number and protein concentration[1].
- Sensitivity comparable with fluorometric assays and better and superior to Lowry or Bradford.
- Excellent signal to noise ratio and the resolution is 1,000-2,000 cells/well[1].
- Suitable for high throughput format.
- Cell labelling is not cell line depending.

Kit Components

Components	TBK0518
Fixing Solution	2x 50 mL
SRB Labeling Dye	0.4 g
Washing Solution 10x	2 x 50 mL
Solubilization Buffer	2 x 100 mL

Order Info Kit Components: *SRB Labeling Dye (TBR0204) | Fixing Solution (TBR0205) | Washing Solution 10x (TBB0563) | Solubilization Buffer (TBB0564).*

Storage

Store the kit at 25°C. Protect from light.

Applications

- Cell viability determination.
- Cytotoxicity.
- Drug toxicity screening.

Quality Control

The kit is tested in a functional assay.

Material required (not supplied)

Distilled Water.

[1] Methods in Molecular Medicine, 2005, 110: 39-48. DOI:10.1385/1-59259-869-2:039.

PROTOCOL

1. Add **200 µL Cell Solution** in a 96-well plate.
   - *For most experiments, a density between $5x10^3$ to 10^6 cells/well in the log phase are optimal. To adjust the appropriate seeding density, use growth medium.*
   - *Include a well with culture medium without cells to use as a background control. As a 100% viability control, either PBS alone or 0.1% DMSO in PBS could be used. As cell inhibition control could be used Doxorubicin 20 mM (1 µL/well).*
2. Incubate at 37°C in 5% CO2 for 72 hours.
3. Prepare conveniently:

Washing Solution 1x	Add **10 mL Washing Solution 10x** to **90 mL Distilled Water**
Labeling Solution	Dissolve **Labeling Dye** tube content in **100 mL Washing Solution 1x**. Protect the solution from light

4. Add **100 µL Fixation Solution** to each well.
5. Incubate 1 hour at 4°C.
6. Add gently **200 µL Distilled Water** and remove by pipetting. Avoid to disturb the cell monolayer.
7. Repeat the previous step three more times.
8. Incubate at 37°C until the plate is dry (~45 minutes).
   After this step and, with the plate dried, it can be stored at room temperature for a month.
9. In the dark, add **100 µL SRB Labeling Solution** to each well.
10. Incubate 30 minutes at room temperature in the dark.
11. Add **100 µL Washing Solution 1x** to each well and remove by pipetting.
12. Repeat the previous step three more times as quickly as possible to avoid bleaching.
13. Dry the plates until there is no visible moisture.
14. Add **200 µL Solubilization Buffer** to each well.
15. Incubate 10 minutes at room temperature with shaking.
16. Read the plate at 565 nm.
    If $A_{565nm} > 3.5$, read the plate at 490-530 nm.
17. To calculate the percentage of cytotoxicity, firstly subtract the Background from all readings. Then calculate the percentage of cytotoxicity as follow

$$\%\ \text{CYTOTOXICITY} = \frac{A_{DMSO} - A_{SAMPLE}}{A_{DMSO}} \times 100\%$$