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Sulforhodamine B Cell Cytotoxicity Assay Kit (Colorimetric)

11/16

(Catalog # K943-1000; 1000 assays, Store kit at -20°C)

I. Introduction:

Sulforhodamine B (SRB) cell cytotoxicity assay is one of the most widely methods used to detect cell viability or drug cytotoxicity. This assay relies on the ability of SRB to bind cellular protein components and measure the total biomass. SRB is a bright-pink aminoxanthene dye that can form an electrostatic complex with basic amino acid residues of proteins in slightly acidic conditions but it can dissociate under basic conditions. It has been widely used for drug toxicity screening against different types of cancerous and non-cancerous cell lines. In addition, this assay is independent of cell metabolic activity and therefore should show less interference by the testing compounds. Since the binding of SRB is stoichiometric, the incorporated dye released from stained cells after washing is directly proportional to the cell biomass and can be measured at 565 nm. BioVision's SRB cell cytotoxicity assay kit is simple, accurate, reproducible and sensitive. This kit offers an excellent and efficient method for *in vitro* cytotoxicity studies as well as high-throughput drug screening that can detect between 5,000-50,000 cells per well.

II. Application:

- *In vitro* cell proliferation cytotoxicity studies
- High-throughput drug screening

III. Sample Type:

- Cell culture: Adherent cells

IV. Kit Contents:

Components	K943-1000	Cap Code	Part Number
Fixation Solution	55 ml	NM	K943-1000-1
20X Washing Solution	50 ml	WM	K943-1000-2
10X Solubilization Solution	22 ml	WM	K943-1000-3
SRB Dye Solution	50 ml	Brown	K943-1000-4
20 mM Doxorubicin	100 µl	Red	K943-1000-5

V. User Supplied Reagents and Equipment:

- 96-well clear well plate
- Multi-well spectrophotometer
- Personal Protective equipment: gloves, goggles, laboratory coat

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. The kit components are stable for one year when stored as recommended. *Wear gloves and goggles when handling 20X washing and fixation solutions*. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment. Bring all reagents to room temperature before use.

- **Washing Solution**: Prepare 1X washing solution by adding 1 part of 20X washing solution to 19 parts distilled water. You may need ~0.8 ml to wash each well. 20X and 1X washing solutions can be stored at 4°C.
- **SRB Dye Solution**: Thaw SRB Dye Solution before use. Stored at -20°C.
- **Solubilization Solution**: Add 1 part of 10X Solubilization Solution to 9 parts distilled water. Store at 4°C.
- **20 mM Doxorubicin:** Thaw doxorubicin before use. After use, doxorubicin should be stored at -20°C.
- **Fixation Solution:** Ready to use. Store at -20°C.

VII. SRB Cytotoxicity Assay Protocol:

1. **Cell Culture**: Grow adherent cells to ~80% confluency. Trypsinize and spin down the cells, add 5 ml of growth medium to disperse the cells. Determine the cell density by using a hemocytometer. Add growth medium to the cells to adjust to an appropriate concentration. Add 200 µl of the cells with a recommended density between 5,000–20,000 cells/well to a 96-well clear flat-bottom plate.
2. **Compound Treatment:** Prepare serial dilutions of your testing compounds appropriately, using DMSO as solvent. Add compounds to the wells. Prepare a DMSO-only well as vehicle control, and another well containing culture medium-only as background control. In addition, add 1 µl of 20 mM doxorubicin to a well containing the cells as an inhibitor control. Incubate the plate at 37°C in a humidified incubator with 5% CO_2 for 72 hr.
3. **Cell Fixation**: Without removing the culture medium, add ¼ volume (eg. 50 µl in 200 µl of culture medium) of the Fixation Solution to the each well. Incubate the plate for 1 hr at 4 °C. Remove the solution and use 200 µl of dH_2O to wash the wells 3 times. *Washing should be done as gentle as possible to avoid disturbance of the cell monolayer*. Remove wash solutions as much as possible by pipetting. After cell fixation, washing and drying steps are complete, the plate can be stored at room temperature for a month if desired.
4. **SRB Staining**: Add 45 µl of SRB Solution to each well and stain for 15 min at room temperature in the dark. *Note that SRB should be protected from light as it is light-sensitive*. After incubation, remove the staining solution. Add 200 µl of 1X Washing Solution to wash each well 4 times. Washing should be done as quickly as possible to avoid bleaching. Remove wash solutions as much as possible by pipetting and air-dry the plate if necessary.
5. **Solubilization:** Add 200 µl of 1X Solubilization Solution to each well. Shake the plate occasionally or place the plate on a shaker for 10 min at room temperature.

6. **Measurement:** Measure the O.D. at 565 nm. If intense color is observed (> O.D. 3.5) due to cell overload you may use a suboptimal wavelength (eg. 490-530 nm) to lower the readings back to the linear range of your instrument.
7. **Calculations:** Correct background by subtracting the O.D. of the control containing only the culture medium (background control well) from all samples readings. Calculate the percentage of cytotoxicity using the formula below:

$$\% \text{ Cytotoxicity} = \frac{O.D._{DMSO} - O.D._{sample}}{O.D._{DMSO}} \times 100\%$$

Where: $O.D._{DMSO}$ is the O.D. of the DMSO control after background correction (corrected negative control well)
$O.D._{sample}$ is the O.D. of the sample after background correction.

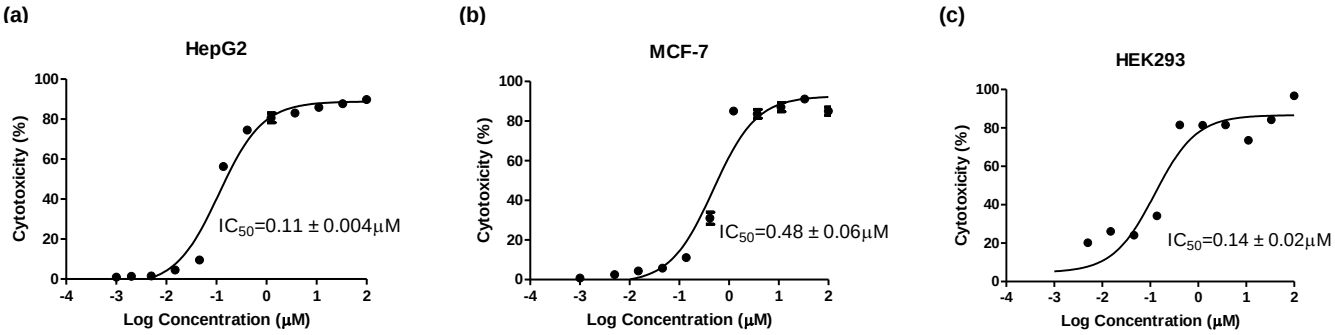

Figure: (a) Dose-response curve of HepG2 (a), MCF-7 (b) and HEK293 (c) cells after exposing to doxorubicin for 72 hr determined by the SRB assay. Assays were performed according to the kit protocol in triplicate.

VIII. RELATED PRODUCTS:

LDH-Cytotoxicity Colorimetric Assay Kit (K311)
Bioluminescence Cytotoxicity Assay Kit (K312)
PicoProbe™ LDH-Cytotoxicity Fluorometric Assay Kit (K314)
MTT Cell Proliferation Assay Kit (Colorimetric) (K299)
MTS Cell Proliferation Colorimetric Assay Kit (K301)
BrdU Cell Proliferation Assay Kit (K306)
StayBrite™ Highly Stable ATP Bioluminescence Assay kit (K791)
LDH-Cytotoxicity Colorimetric Assay Kit II (K313)
Senescence Detection Kit (K320)
PicoProbe™ Lactate Dehydrogenase Activity Assay Kit (K730)
ADP Colorimetric/Fluorometric Assay Kit (K355)
ATP Colorimetric Assay Kit II (K354)
ApoSENSOR™ ADP/ATP Ratio Bioluminescence Assay Kit (K255)
ApoSENSOR™ ATP Cell Viability Bioluminescence Assay Kit (K254)

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