Version 3a, Last updated 7 June 2023

ab235935 Sulforhodamine B Cell Cytotoxicity Assay Kit (Colorimetric)

For the measurement of cytotoxicity in cultured adherent cells.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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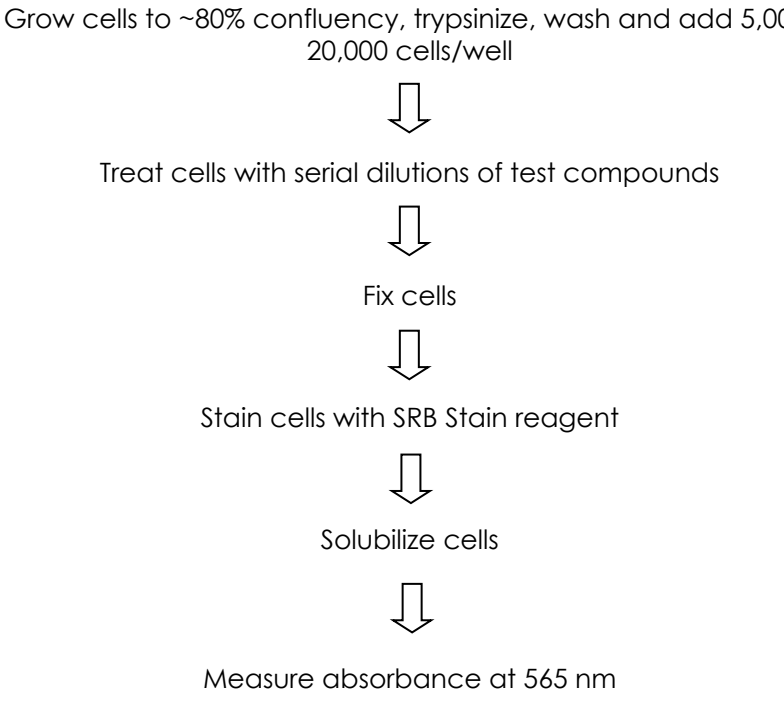
1. Overview

Sulforhodamine B Cell Cytotoxicity Assay Kit (Colorimetric) (ab235935) 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.



2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Fixation Solution II/Fixation Solution	55 mL	-20°C	-20°C
20X Wash Buffer/Washing Solution (20X)	50 mL	4°C or -20°C	4°C or -20°C
10X Solubilization Solution/Solubilization Solution (10X)	22 mL	-20°C	4°C
SRB Solution/SRB Dye Solution	50 mL	-20°C	-20°C
Doxorubicin/Doxorubicin (20 mM)	100 µL	-20°C	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well clear well plate
- Multi-well spectrophotometer capable of reading absorbance at 565 nm
- Personal Protective equipment: gloves, goggles, laboratory coat

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

ΔNote: Wear gloves and goggles when handling 20X washing and fixation solutions.

5.1 20X Wash Buffer/Washing Solution (20X)

1. Prepare 1X wash buffer/washing solution by adding 1 part of 20X Wash Buffer/20X washing solution to 19 parts distilled water.
2. You may need ~0.8 ml to wash each well.
3. 20X and 1X wash buffer/washing solutions can be stored at 4°C.

5.2 SRB Solution/SRB Dye Solution

1. Ready to use as supplied.
2. Thaw SRB Solution/SRB Dye Solution before use. Store at -20°C.

5.3 10X Solubilization Solution/Solubilization Solution

1. Add 1 part of 10X Solubilization Solution/10X Solubilization Solution to 9 parts distilled water.
2. Store at 4°C.

5.4 Doxorubicin/Doxorubicin (20 mM)

1. Ready to use as supplied.
2. Thaw doxorubicin before use. After use, doxorubicin should be stored at -20°C.

5.5 Fixation Solution II/Fixation Solution

1. Ready to use as supplied.
2. Store at -20°C.

6. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

6.1 Cell culture:

1. Grow adherent cells to ~80% confluency.
2. Trypsinize and spin down the cells, add 5 ml of growth medium to disperse the cells.
3. Determine the cell density by using a hemocytometer. Add growth medium to the cells to adjust to an appropriate concentration.
4. 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.
5. Add only cell culture medium, no cells, to at least one well. This control will account for SRB dye binding directly to the wells.

6.2 Compound treatment:

1. Prepare appropriate serial dilutions of your test compounds using DMSO as solvent.
2. Add DMSO to at least one cell-seeded well for a vehicle control. This well should contain the same amount of DMSO as the wells that receive treatments prepared in DMSO. This will account for any cytotoxic effect of DMSO.
3. Add 1 µL of Doxorubicin/20 mM doxorubicin to a well containing the cells as an inhibitor control.
4. Incubate the plate at 37°C in a humidified incubator with 5% CO_2 for 72 hours.

6.3 Cell fixation:

1. Without removing the culture medium, add ¼ volume (eg. 50 µl in 200 µL of culture medium) of the Fixation Solution II/Fixation Solution to the each well. Incubate the plate for 1 hour at 4 °C.
2. Remove the solution and use 200 µL of dH_2O to wash the wells 3 times. **Washing should be done as gently as possible to avoid disturbance of the cell monolayer.** Remove wash solutions as much as possible by pipetting.

3. After cell fixation, washing and drying steps are complete, the plate can be stored at room temperature for a month if desired.

6.4 SRB Staining:

1. Add 45 µl of SRB Solution to each well and stain for 15 minutes at room temperature in the dark.

ΔNote: SRB should be protected from light as it is light-sensitive.

2. After incubation, remove the staining solution. Add 200 µL of 1X Wash Buffer/Washing Solution to wash each well 4 times. Washing should be done as quickly as possible to avoid bleaching.
3. Remove wash solutions as much as possible by pipetting and air-dry the plate if necessary.

6.5 Solubilization:

1. Add 200 µL of 1X Solubilization Solution to each well.
2. Shake the plate occasionally or place the plate on a shaker for 10 minutes at room temperature.

6.6 Measurement:

1. Measure the absorbance at 565 nm.
2. 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. Data Analysis

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (containing only culture medium) from all controls and sample readings. This is the corrected absorbance.
3. Calculate the percentage cytotoxicity as follows:

$$\text{Cytotoxicity (\%)} = \frac{\text{O.D.(DMSO) - O.D. (Sample)}}{\text{O.D. (DMSO)}} * 100\%$$

Where:

O.D. (DMSO) = O.D. of the DMSO control after background correction (corrected negative control well).

O.D. (Sample) = O.D. of the sample after background correction.

8. FAQs / Troubleshooting

General troubleshooting points are found at
www.abcam.com/assaykitguidelines

9. Typical Data

Data provided **for demonstration purposes only.**

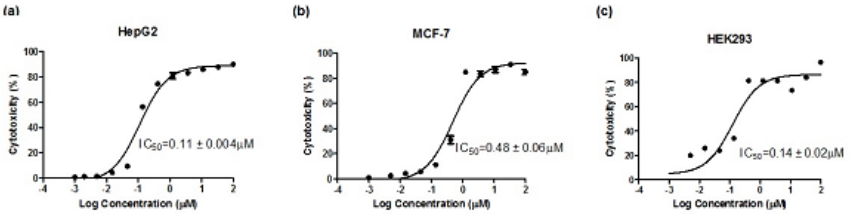


Figure 1. Dose-response curve of HepG2 (a), MCF7 (b) and HEK-293 (c) cells, after exposure to doxorubicin for 72 hours, as determined using the Sulforhodamine B Cell Cytotoxicity Assay Kit (Colorimetric). Assays were performed according to the kit protocol in triplicate.

10. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)