SRB Cell Proliferation and Cytotoxicity Assay Kit

Introduction

SRB Cell Proliferation and Cytotoxicity Assay Kit is a kit that uses sulforhodamine B (SRB) to measure cell viability and toxicity. The detection principle of this kit is based on that SRB dyes can bind to the basic amino acids of proteins in cells and form stable complexes. The complex has specific absorption at 515 nm, and its absorbance is proportional to the number of cells.

Compared with traditional MTT assays, this kit is not only easier to use, but also more sensitive, so it is widely used in cytotoxicity testing, drug screening, and monitoring of cell proliferation and viability.

Components and Storage

Components \ Size	500 Tests	Storage
Fixation Solution	25 mL	4°C
Wash buffer I	100 mL	4°C
SRB Staining Solution	25 mL	-20°C away from light
Wash buffer II (2×)	500 mL	4°C
Solubilization Solution	50 mL	4°C
Doxorubicin (20 mM)	100 µL	-20°C
Shipping: Blue ice	Shelf life: 18 months	

Protocol

1. **Cell culture:** For 96-well plates, seed cells at a density of 5-100x10^3 cells/well in 100 µL culture medium. Treat cells with the interested drug for a desired period. Prepare parallel wells as the background control (only containing medium) and negative control (containing medium, cells and the same volume of solvent for the interested drug).

***Note:** The optimal number of cells seeded in each well varies depending on the cell types.

2. **Cell fixation:** Remove the culture medium, and add 50 µL of Fixation Solution per well. Then Incubate at 4°C for 1 h.

***Note:** The fixative solution is toxic, and it is recommended to take safety precautions.

3. **Cell Washing:** Remove the Fixation Solution, and add 100 µL of Wash buffer I per well. Then stand at room

temperature for 30 s, and remove the Wash buffer I. Repeat the step once time.

4. **SRB Staining:** After washing, remove Wash buffer I, and add 50 μL SRB Staining Solution per well. Then incubate at room temperature in the dark for 15 min. At this time, dilute an appropriate amount of Wash buffer II (2×) with sterilized water to make a Wash buffer II (1×) according to the needs of the experiment.

***Note:** The optimal incubation time varies depending on the cell types.

5. **Cell Washing:** Remove the SRB Staining Solution, and add 200 μL of Wash buffer II (1×) per well to wash 4-10 times. Washing should be done as quickly as possible to avoid bleaching.

6. **SRB Solubilization:** Remove the Wash buffer II (1×), and add 100 μL of Solubilization Solution per well. Then incubate at room temperature in the dark for 5 min.

***Note:** For better solubilization of SRB, 96-well plates can be placed on a shaker for incubation.

7. **Detection:** Measure the absorbance (A) at 515 nm with a microplate reader.

8. **Analysis:** Calculate cell viability with the following equation

$$\text{Cell viability (\%)} = [(A_{\text{Treatment sample}} - A_{\text{Background control}}) / (A_{\text{Negative control}} - A_{\text{Background control}})] \times 100$$

9. **Positive control (optional):** Doxorubicin is provided as a positive inhibitor in this kit. If used, Doxorubicin (20 mM) can be diluted with DMSO into different gradient concentrations, typically 7-9 concentrations. Treat cells with different concentrations of Doxorubicin for 48-72 h. After the treatment, detect the cell proliferation with this kit and fit the IC_{50}.

Note

1. When cells are cultured for long periods, the corner and edge wells of the 96-well plate are prone to liquid evaporation. It is recommended to fill the surrounding moat with sterile water, medium, or PBS. Meanwhile, place the plate near the water source in the incubator.

2. SRB Staining Solution needs to be mixed before use, and if precipitation occurs, it can be filtered before use.

3. For your safety and health, please wear lab coats and gloves during the experiment.

4. For research use only. Not to be used in clinical diagnostic or clinical trials.

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