Neutral Red Cell Proliferation Assay Kit

Introduction

Neutral Red Cell Proliferation Assay Kit is a kit that can quickly detect cell proliferation or toxicity. The uptake of neutral red by cells is determined by the ability of cells to maintain a pH gradient, which is dependent on ATP production. Under physiological pH conditions, neutral red can enter the cell membrane by passive diffusion and accumulate in lysosomes. When the number of cells increases, the intake of neutral red also increases. Conversely, when cells are damaged, their ability to ingest neutral red is diminished. By measuring the amount of neutral red ingested by the cells, the proliferation or toxicity of the cells can be assessed.

In addition, neutral red can also be used as a pH indicator, and its color will change with the pH value. Neutral red appears red in acidic environments, and gradually changes color from red to yellow when the pH rises from 6.8 to 8.0.

Components and Storage

Components	K2262-500T
Neutral Red Solution	10 mL
Neutral Red Lysis Buffer	50 mL
Store the kit at -20°C, stable for 1 year. Neutral Red Solution should be stored away from light.	

Protocol

1. **Ready-to-use lysis buffer preparation:** Add 50 mL of absolute ethanol to the bottle of Neutral Red Lysis Buffer and shake to make the ready-to-use lysis buffer.
2. **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.

3. **Neutral red intake:** Prepare the neutral red staining solution referring to the table below. Remove the medium, add the freshly prepared neutral red staining solution, and incubate in the incubator for 2 h. The incubation time can be adjusted according to the specific experiment and can be extended to 3-4 h.

Samples in 96 well plate	1	2	3
Neutral Red Solution	20 μL	40 μL	60 μL
Culture medium	185 μL	370 μL	555 μL

4. **Neutral red lysis:** Remove medium and wash 1-2 times with PBS. Add 200 μL of ready-to-use lysis buffer and lyse on a shaker at room temperature for 10 minutes.
5. **Detection:** Measure the absorbance (A) at 540 nm with a microplate reader. A wavelength of 690 nm can be selected as the reference wavelength.
6. **Analysis:** Calculate cell viability with the following equation

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

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. Long-term storage of the Neutral Red Solution may produce precipitation, which can be performed directly by removing the supernatant for experimentation or filtering before use. This does not affect the detection as the neutral red in the solution is excessive.
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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