Cell Counting Kit(Neutral Red Method)

Cat: G1317
Size: 500T/1000T
Storage: 2-8℃, avoid light, valid for 1 year.

Kit Components

Reagent	500T	1000T	Storage
Reagent(A)：CCK-N Stain Solution	5mL	10mL	2-8℃, avoid light
Reagent(B)：CCK-N Extract Solution	50mL	100mL	2-8℃

Introduction

The uptake of neutral red by cells depends on the ability of living cells to maintain pH gradients. Under physiological pH conditions, the net charge of neutral red dye is almost zero, allowing it to penetrate the cell membrane and enter the cell through non ionic passive diffusion. The proton gradient in lysosomes causes the pH value in the lysosomes to be lower than the cytoplasm, which can charge neutral red and accumulate in the lysosomes. When cell proliferation accelerates, the number of cells increases, and the amount of neutral red that can be consumed will increase. When cells are damaged, the ability to absorb neutral red decreases. After a certain period of intake, the cells are washed and lysed with lysate to release neutral red for detection. By measuring the intake of neutral red by cells, the proliferation or toxicity of cells can be determined.

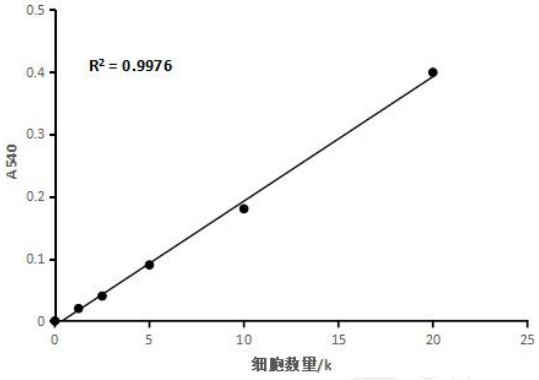
The Cell Counting Kit(Neutral Red Method) is a kit that detects cell proliferation or cytotoxicity based on the ability of cells to absorb neutral red. This product has stable properties, is not easy to precipitate, and has good repeatability.

Protocol(*for reference only*)

1. Add 100μl of cell culture medium to each well of the 96 well plate, set appropriate negative and positive controls, and administer medication stimulation.(See Note 4)
2. At the time point to be tested, if it is speculated that the drugs in the culture medium will not interfere with subsequent testing, directly add 10ul of CCK-N Stain Solution; If the drugs in the culture medium may interfere with subsequent detection, first wash with 1×PBS 1-2 times, then add 100ul of cell culture medium and 10ul of CCK-N Stain Solution.Alternatively, CCK-N Stain Solution can be directly mixed into the culture medium and added to the well plate.
3. Incubate the cells in a constant temperature incubator for 2 hours. (See Note 2)
4. Remove the cell culture medium containing CCK-N Stain Solution and wash it 1-2 times with 1×PBS.
5. Add 100ul of CCK-N Extract Solution and crack at room temperature for 10 minutes. Shaking on a shaking table can promote the cracking and extraction of the sample.
6. When measuring the absorbance value of the liquid after cracking at 540nm, 690nm can be selected as the reference wavelength.

Result

The volume of the vacuolar system of a single cell tends to be consistent, that is, within a certain range of cell numbers, the amount of neutral red that can be absorbed is linearly correlated with the number of cells. By bringing in the standard curve, digital statistics can be conducted on the changes in cell numbers caused by proliferation and toxic damage. Taking A549 cells as an example, the detection results are as follows:



Note

1. When using this reagent kit for the first time, it is recommended to take a small amount of samples for preliminary experiments. The measured data of this product may vary due to differences in cell type, cell status, testing instruments, etc.

2. For situations where cell density is very low and cell metabolism rate is very slow, the incubation time can be extended to 3-4 hours.
3. Long term storage of CCK-N Stain Solution may result in precipitation. The supernatant of the staining solution can be extracted by centrifugation for use, or a needle filter can be used to filter and remove sediment before continuing use, without affecting the effectiveness of use. Because the neutral red in the staining solution is already excessive.
4. During the cell culture process, there may be evaporation issues with the 96 well plate. 100ul of PBS can be added to each well in the outer circle to prevent evaporation and avoid interference with subsequent neutral red absorption and data detection.
5. For your safety and health, please wear laboratory clothes and disposable gloves for operation.