Cell Counting Kit-8

Cat: CA1210
Size: 100T/500T/1000T/10*500T/10*1000T
Storage: 2-8℃, avoid light, valid for 1 year。

Kit Components

Reagent	100T	500T	1000T	10*500T	10*1000T
Cell Counting Kit-8	1mL	5mL	10mL	10*5mL	10*10mL

Introduction

The Cell Counting Kit-8(CCK-8 Kit) is a fast and highly sensitive cell activity detection kit based on WST-8, widely used for cell proliferation and cytotoxicity detection. WST-8 is a compound similar to MTT that can be reduced by some dehydrogenases in mitochondria in the presence of electron coupling reagents to produce orange yellow water-soluble formaldehydes. The more cells proliferate, the faster the color becomes; The greater the cytotoxicity, the lighter the color. For the same type of cells, there is a linear relationship between the color depth and the number of cells within a certain limit.

This kit is a ready-to-use solution that does not require further preparation and can be directly used for 96 well plate cell experiments, omitting steps such as cell washing and collection. This kit can be used for cell proliferation detection induced by cytokines, cytotoxicity detection induced by cytotoxic agents such as anticancer drugs, or cell growth inhibition detection induced by some drugs.

Protocol(*for reference only*)

Making standard curves

1. First, count the number of cells in the prepared cell suspension using a cell counting chamber, and then inoculate the cells.
2. Dilute the culture medium proportionally in order to form a cell concentration gradient. Generally, 3-5 cell concentration gradients are required, with 3-6 wells in each group for average value.
3. After inoculation, culture until the cells adhere to the wall, and then add Cell Counting Kit-8 to culture for a certain time. Measure the OD value, and create a standard curve with the number of cells as the horizontal axis (X-axis) and the OD value as the vertical axis (Y-axis).
4. According to this standard curve, the number of cells in unknown samples can be determined (the premise for using this standard curve is that the experimental conditions must be consistent, which is convenient for determining the number of cells inoculated and the cultivation time after adding Cell Counting Kit-8.)

Cell activity detection

1. Inoculate cell suspension (100μL/well) in a 96 well plate. Place the culture plate in an incubator for pre cultivation (at 37 ℃, 5% CO2).
2. Add 10μL of Cell Counting Kit-8 to each well (be careful not to generate bubbles in the well as they may affect the reading of the OD value).
3. Incubate the culture plate in the incubator for 1-4 hours.
4. Measure the absorbance at 450nm using an enzyme-linked immunosorbent assay.
5. If the OD value is not measured temporarily, you can add 10μL of 0.1M HCl or 1% SDS (W/V) solution to each well, and the culture plate can be covered and stored in dark at room temperature. The absorbance will not change within 24 hours.

Cell proliferation toxicity detection

1. Prepare 100μL of cell suspension in a 96 well plate. Pre cultivation the culture plate in the incubator for 24 hours (at 37 ℃, 5% CO2).
2. Add 10μL of different concentrations of the substance to be tested to the culture plate. Incubate in the incubator for a period of time (e.g. 6, 12, 24, or 48 hours).
3. Add 10μL of Cell Counting Kit-8 to each well (be careful not to generate bubbles in the well as they may affect the reading of the OD value). If the substance to be tested has oxidizing or reducing properties, fresh culture medium can be replaced before adding Cell Counting Kit-8 (remove the culture medium, wash the cells twice with the culture medium, and then add a new culture medium) to remove the drug effect.
4. Incubate the culture plate in the incubator for 1-4 hours, and measure the absorbance at 450nm using an enzyme-linked immunosorbent assay.
5. If the OD value is not measured temporarily, you can add 10μL of 0.1M HCl or 1% SDS (W/V) solution to each well, and the culture plate can be covered and stored in dark at room temperature. The absorbance will

not change within 24 hours.

Vitality calculation

cell viability(%)=[A(dosing)－A(blank)] / [A(0 dosing)－A(blank)]×100

A(dosing)：Absorbance of wells with cells, Cell Counting Kit-8, and drug solution

A(blank)：Absorbance of wells with culture medium and Cell Counting Kit-8 without cells

A(0 dosing)：Absorbance of wells with cells and Cell Counting Kit-8 without drug solution

Cell viability:cell proliferation activity or cell cytotoxic activity

Note

1. The color of this reagent should be pink, and if the solution turns yellow, it should be discarded.
2. Due to the use of 96 well plates for detection, it is important to pay attention to evaporation issues because of long culture time . Due to the fact that the area around the 96 well plate is most prone to evaporation, can discard the surrounding area and add the same amount of PBS, water, or culture medium instead of cells.
3. The kit relies on dehydrogenase-catalyzed reactions, so reducing agents (such as some antioxidants) may interfere with the detection. If there are many reducing agents in the system to be tested, it is necessary to try to remove them.
4. It is recommended to first make a few holes to explore the number of inoculated cells and the cultivation time after adding Cell Counting Kit-8. White blood cells may take longer to cultivate.
5. When using a standard 96 well plate, the minimum inoculation amount for adherent cells is at least 1000 cells per well(100μL culture medium). The sensitivity of detecting white blood cells is relatively low, so it is recommended to inoculate at least 2500 cells per well (100μL culture medium). If you want to use a 24 well or 6 well plate experiment, please first calculate the corresponding inoculation amount for each well and add Cell Counting Kit-8 at 10% of the total volume of culture medium for each well.
6. If there is no 450nm filter, a filter with an absorbance between 430-490nm can be used, but 450nm has the highest detection sensitivity.
7. The absorbance of phenol red in the culture medium can be eliminated by subtracting the absorbance of the background in the blank well during calculation, so it will not affect the detection.