Ver. EN20230302

Cell Counting Kit (CCK-8)

Product description

Cell Counting Kit-8 (CCK-8) is a fast and highly sensitive detection kit based on the WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt) reagent. WST-8 is an upgraded reagent of MTT and can be reduced to a highly water-soluble orange-yellow formazan by dehydrogenases in the mitochondria. The amount of the formazan generated is proportional to the number of living cells. The OD value of formazan at 450nm detected by a microplate reader can indirectly indicate the number of viable cells. This kit is widely used for drug screening, cell proliferation and cytotoxicity assays, tumor drug sensitivity testing, and activity detection of biological factors.

Components

Components No.	Name	40203ES60	40203ES76	40203ES80	40203ES88	40203ES92
40203	Cell Counting Kit (CCK-8)	100 T	500 T	1000 T	3×1000 T	10×1000 T

Storage

The product could be stored at -25~-15℃ in a dry and dark place for two years.

Instructions

I. Make a standard curve

1. Prepare the cell suspension, determine the cell density, and then plate cells.
2. Sequentially dilute the cells with the culture medium according to a certain ratio (such as 1:2 ratio) to form a cell concentration gradient, generally 3-5 cell concentration gradients, 3-6 replicate wells for each concentration.
3. After plated, culture the cells for 2-4 hours to make them adhesion. Then, add the CCK-8 reagent, incubate for a certain period of time and measure the OD value. Plot a standard curve with the cell number as the X-axis and the OD value as the Y-axis. According to this standard curve, the cell number in an unknown sample can be determined (The premise of using this standard curve is that the experimental conditions should be consistent).

II. Cell viability assay

1. Plate cells (100 µL/well) in a 96-well plate. Place the plate in an incubator (37°C, 5% CO_2) for a period of time for pre-incubation.
2. Add 10 µL of CCK-8 reagent to each well (be careful not to introduce bubbles to the wells, since they interfere with the OD reading) and mix gently.
3. Place the plate in the incubator and incubate for 1-4 hours.
4. Measure the absorbance at 450 nm with a microplate reader.
5. If the OD value is not measured immediately, add 10 µL of 0.1 M HCl solution or 1% w/v SDS solution to each well, cover the plate and store it with protection from light at room temperature. The absorbance value will not change within 24 hours.

III. Cell proliferation and cytotoxicity assay

1. Plate cells (100 µL/well) in a 96-well plate. Place the plate in an incubator (37°C, 5% CO_2) for 24 hours for pre-

incubation.

2. Add 10 μL of different concentrations of the substance to be tested to the plate.

3. Place the plate in the incubator and incubate for a certain period of time (such as 6, 12, 24, or 48 hours).

4. Add 10 μL of CCK-8 reagent to each well (be careful not to introduce bubbles to the wells, since they interfere with the OD reading) and mix gently.

5. Place the plate in the incubator and incubate for 1-4 hours.

6. Measure the absorbance at 450 nm with a microplate reader.

7. If the OD value is not measured immediately, add 10 μL of 0.1 M HCl solution or 1% w/v SDS solution to each well, cover the plate and store it with protection from light at room temperature. The absorbance value will not change within 24 hours.

Note: If the substance is oxidizing or reducing, replace the old culture medium with fresh medium (remove the old medium, wash the cells twice with fresh medium, and then add fresh medium) before adding the CCK-8 reagent to eliminate the effect of the substance. If the substance itself has only a slight effect on the absorbance value, there is no need to replace the culture medium and the effect of the substance can be eliminated by subtracting the absorptance value of a blank group with the substance.

Calculation formula

Cell survival rate =[(A-C) /(B-C)] x100%

Inhibition rate =[(B-A) /(B-C)] x100%

A: The absorbance of the experimental group (the absorbance of the culture medium containing cells, CCK-8 reagent, and the substance to be tested)

B: The absorbance of the control group (the absorbance of the culture medium containing cells, CCK-8 reagent)

C: The absorbance of blank group (the absorbance of the culture medium containing CCK-8 reagent)

Precautions

1. In the first experiment, it is recommended to determine the optimal number of cells plated and the optimal incubation time of CCK-8 reagent.

2. If possible, use a multi-channel pipette to reduce variations between replicate wells. Avoid bubbles in the experiment as bubbles will interfere with the OD reading. When adding the CCK-8 reagent, it is recommended to add it close to the wall of the culture plate rather than inserting it into the culture medium.

3. White blood cells may require a longer incubation time.

4. When using a standard 96-well plate, the number of cells plated is at least 1,000 cells/well (100 μL). The detection sensitivity for white blood cells is relatively low, so it is recommended to plate at least 2,500 cells/well (100 μL). If using a 24-well or 6-well plate, calculate the corresponding number of cells for each well and add the appropriate volume of CCK-8 reagent (the volume of CCK-8 reagent added is 10% of the volume of the culture medium in each well of the plate).

5. If the 450 nm filter is not available, a filter with absorbance between 430-490 nm can be used, but the 450 nm filter can achieve the highest detection sensitivity.

6. Phenol red does not affect the measurement as the absorbance of phenol red in the medium can be eliminated by subtracting the absorbance of the blank group.

7. For your safety and health, please wear the lab coat and disposable gloves when operating the experiment.