Super Sensitivity Cell Counting Kit-8 (CCK-8)

Cat #: D-AKE106

Size: 1000T / 10000T

Storage: Stored at 4°C for 12 months, and at -20°C for at least 24 months, protected from light

Assay Principle

Super Sensitivity Cell Counting Kit-8 (CCK-8) allows very convenient assays by utilizing highly water-soluble tetrazolium salt-WST-8. WST-8 is reduced by dehydrogenases in cells to give an orange colored product (formazan), which is soluble in the tissue culture medium. CCK-8, being nonradioactive, allows sensitive colorimetric assays for the determination of the number of viable cells in cell proliferation and cytotoxicity assays. The amount of the formazan in cells is directly proportional to the number of living cells, which can be used to detect cell proliferation and cell toxicity. The detection sensitivity using CCK-8 is higher than assays using other tetrazolium salts such as MTT, XTT, MTS or WST-1.

Materials Supplied and Storage Conditions

Kit components	Size（1000T）	Size（10000T）	Storage conditions
Ready-to-use CCK-8 solution	10 mL	100 mL	4°C, -20°C (long time)

Materials Required but Not Supplied

·Microplate reader capable of measuring absorbance at OD_{450} nm

·Humidifying carbon dioxide incubator (37°C， 5% CO_2）

·96-well cell culture plate with clear flat bottom, precision pipettes, disposable pipette tips

Reagent Preparation

Ready-to-use CCK-8 solution: Ready-to-use, no premixing of components required.

Assay Procedure

Note: Before the actual experiment, do a pre-experiment to determine the appropriate number of cells and incubation time. If the OD value is too high, the number of cells can be appropriately reduced, the incubation time of adding CCK-8 can be shortened, or the loading amount of CCK-8 can be reduced.

I Drawing of standard curve

1. Count the number of cells in the prepared cell suspension with a cytometer, and then inoculate the cells.

2. Dilute the cell with the medium to form a cell concentration gradient in sequence, usually 5-7 cell concentration gradients, 4-6 multiple wells per concentration gradient.

3. After inoculation, incubate the adherent cells or suspended cells for 0.5-4 h, add 10 μL CCK-8 reagent per 100 μL medium to incubate for a certain period of time and then measure the OD_{450} value. With the number of cells as the x-axis and the OD_{450} value as the y-axis, draw the standard curve. According to this standard curve, the number of cells in unknown samples can be determined. The prerequisite for using this standard curve is that the test conditions are exactly the same.

II Cell Activity Assay Protocol

1. Inoculate cell suspension (100 μL/well) in a 96-well plate. Pre-incubate the plate in a humidifying carbon dioxide incubator.

2. Add 10 μL CCK-8 solution to each well of the plate. Be careful not to introduce bubbles to the wells, since they interfere with the OD reading.

3. Incubate the plate for 0.5-4 h in the incubator. The incubation time depends on the experimental conditions such as cell type and cell density.

4. Measure the absorbance at 450 nm using a microplate reader.

III Cell Proliferation and Cytotoxicity Assay Protocol

1. Dispense 100 μL of cell suspension (5,000 cells/well) in a 96-well plate. Pre-incubate the plate for 24 h in a humidifying carbon dioxide incubator.

2. Add 1-10 μL of various concentration of substances to be tested to the plate.

3. Incubate the plate for an appropriate length of time (e.g.,6, 12, 24 or 48 h) in the incubator.

4. Add 10 μL of CCK-8 solution to each well of the plate. Be careful not to introduce bubbles to the wells, since they interfere with the OD reading.

5. Incubate the plate for 0.5-4 h in the incubator. The incubation time depends on the experimental conditions such as cell type and cell density.

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

Data Analysis

Cell viability=$[(A_s-A_b)/(A_c-A_b)]\times100\%$

Inhibition rate=$[(A_c-A_s)/(A_c-A_b)]\times100\%$

A_s: Absorbance of experimental wells (including cells, culture medium, CCK-8 solution and drug solution);

A_c: Absorbance of control wells (including cells, culture medium, CCK-8 solution, without drug);

A_b: Absorbance of blank wells (including culture medium, CCK-8 solution, without cells and drugs).

Precautions

1. The cells after CCK-8 detection can be used for other cell assays because of the low toxicity of CCK-8.

2. If the OD value is too low, please increase the number of cells appropriately or extend the incubation time after adding CCK-8.

3. The kit relies on the reaction catalyzed by dehydrogenase and conditions or chemicals that affect dehydrogenase activity in living cells will interfere with the detection. If the color or pH of the medium is changed due to long-term cultivation, please change the medium when adding CCK-8.

FAQ

1. This product is sensitive and OD value is occasionally high. How should we deal with it?

Make adjustments in three aspects: (1) Use different number of cells for detection, make standard curve, and determine the appropriate number of cells; (2) Shorten the incubation time of CCK-8; (3) Reduce the loading amount of CCK-8 to dilute CCK8 to an appropriate concentration for use.

2. How to determine the detection range of this product?

It is suggested to make a standard curve with different cell numbers and determine the detection interval of specific cells according to the stable linear trend.

3. How to set up cytotoxicity tests?

There are two ways to set it:

(1) Add 1-10 μL of various concentrations of substances to be tested into 100 μL cell suspension, incubate for an appropriate time, and then add 10% of the solution in the system.

(2) After the drug exposure to cells for a period of time,10% CCK-8 is used to detect cell proliferation.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.