Cell Counting Kit-8

Catalog No.: EZB-CK8

Description

The *EZ*Bioscience® Cell Counting Kit-8(CCK-8) is a highly sensitive, non-radioactive colorimetric detection kit, which is based on WST-8 and used to determine the number of living cells in cell proliferation or toxicity experiments. In the presence of 1-methoxy PMS, WST-8 can be reduced to highly water-soluble orange-yellow formazan by intracellular dehydrogenase. The amount of the formazan generated is proportional to the number of living cells. Therefore, the number of living cells can be measured by measuring the absorbance of the formazan at 450 nm. CCK-8 Reagent of this kit is a ready to use reagent, which can be directly added to the cell samples, and can be detected after incubation for a certain period of time without pre-preparing various components.

Components

Components	EZB-CK8-S 100 rxns	EZB-CK8 500 rxns	EZB-CK8-L 3000 rxns
CCK-8 Reagent	1 ml	5 ml	30 ml

Note: Use 10 µl per reaction

Storage

The kit is recommended to be stored at 2 ~ 8°C and protected from light.

Precautions

1. In the first experiment, it is recommended to find the optimal number of cells plated and the optimal incubation time of CCK-8 Reagent (normal incubation time is 1 - 4 h).

2. Avoid bubbles in the experiment. When adding CCK-8 Reagent, it is recommended to add it close to the culture plate wall instead of inserting it into the culture medium.

3. The detection of this kit depends on the reaction catalyzed by dehydrogenase, so reducing substances and oxidizing substances will interfere with the detection. Please remove the interfering substances before using CCK-8 Reagent for detection.

4. When conducting drug inhibition experiments, if the drug contains metals ($PbCl_2$, $FeCl_2$, $CuSO_4$, etc.), it will affect the reaction of CCK-8 Reagent, and ultimately lead to a decrease in the sensitivity of detection.

5. If you want to determine the specific number of cells, it is recommended to make a standard curve first.

User-supplied materials

Multi-channel pipette (10 ul, 100 - 200 ul), Microplate reader capable of measurement at or near 450 nm, 96-well cell culture plate, CO_2 incubator

Protocol

1. Make a standard curve

(1) Prepare the cell suspension, determine the cell density, and then plate cells.

(2) According to the ratio (such as 1:2 ratio), sequentially dilute with the medium to form a cell concentration gradient, generally 5 - 7 cell concentration gradients, 3 - 6 replicate wells in each group, plated into a 96-well plate, then add 100 ul of cell suspension to each well.

(3) After plated, incubate for 2 - 4 hours to make the cells adhesion, then add 100 ul of CCK-8 Reagent to each well, mix gently, and incubate in a 37°C incubator for a certain period of time (depending on different cell types, generally 1 - 4 h), use a microplate reader to measure the absorbance at 450 nm to create a standard curve with the number of cells as the abscissa (X axis) and the absorbance as the ordinate (Y axis). According to this standard curve, the number of cells in unknown samples can be determined (The premise of using this standard curve is that the experimental conditions should be consistent).

2. Cell viability detection

(1) Plate cells (100 ul /well) in a 96-well plate. Place the plate in an incubator for pre-incubation (under 37°C, 5% CO_2), and set up a control group and a blank group at the same time.

(2) Add 10 ul of CCK-8 Reagent to each well (it is recommended to add along the wall to avoid bubbles), and mix gently.

(3) Place the plate in the incubator and incubate for a certain period of time.

(4) Measure the absorbance at 450 nm with a microplate reader.

3. Cell proliferation-toxicity assay

(1) Plate cell suspension (100 ul /well) in a 96-well plate. Place the plate in an incubator for pre-incubation for 24 h (under 37°C, 5% CO_2), and set up a control group and a blank group at the same time.

(2) Add 10 ul of different concentrations of the substance to be tested to the plate.

(3) Place the plate in the incubator and incubate for an appropriate period of time.

(4) Add 10 ul CCK-8 Reagent to each well (it is recommended to add along the wall to avoid bubbles) and mix gently.

(5) Place the plate in the incubator and incubate for a certain period of time.

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

4. Calculation formula

Cell survival rate = [(A-C) / (B-C)] ×100%

Inhibition rate = [(B-A) / (B-C)] ×100%

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

B: The absorbance of the control group (the absorbance of the cell culture medium containing cells, CCK-8 Reagent, and no test substance)

C: The absorbance of blank group (the absorbance of the cell culture medium and CCK-8 Reagent)

Representative Results

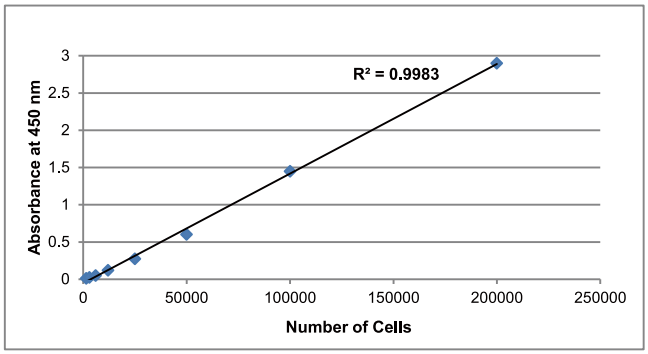

Figure 1. There is a linear relationship between the absorbance and the number of cells. Plate 293T cells (100 ul/well) in a 96-well microplate, place the plate in an incubator for 4 hours. Then add 10 ul CCK-8 Reagent to each well, incubate the plate in an incubator for 1 h, and measure the absorbance at 450 nm with a microplate reader.

Additional Considerations

1. How many cells should be plated in each well?

When 96-well plates were used, the minimum plated number of adherent cells is at least 1,000 cells / well (100 μl cell culture medium). The plated number of WBC is not less than 2,500 cells / well (100 μl cell culture medium).

2. Is CCK-8 Reagent toxic to cells?

CCK-8 Reagent has very low cytotoxicity and does not affect cell growth. Therefore, the same cells can also be used in other detection experiments, such as neutral red stain or crystal violet stain.

3. During the experiment, the 96-well plate is not used to process the cells. What is the volume of CCK-8 Reagent added?

The added volume of CCK-8 Reagent is 10% of the volume of the cell culture medium in each well of the plate, and it can be converted according to this ratio. If you use a 384-well plate for experiment, it is recommended that CCK-8 Reagent is dilute by 1 time with ddH_2O, and then add 20% of the volume of each well of the plate.

4. Which substances will affect the determination of CCK-8?

In the presence of reducing substances, it will affect the determination of CCK-8 and increase the absorbance; In the presence of oxidizing substances, it will inhibit the reaction and reduce the absorbance; In the presence of phenol red, it will increase the blank absorption, but it will not affect the determination, and the blank absorption can be deducted.

5. Does the drug have any effect on the determination in the dosing experiment? How to solve it?

Sometimes it has an impact. If the drug is reductive, it will react with CCK-8 Reagent to increase the absorbance. Solution: first of all, confirm whether the drug has absorption. Add CCK-8 Reagent to the medium containing the drug and measure the absorbance value at 450 nm. If its absorbance is higher than that of the medium without the drug (with CCK-8 Reagent), it is proved that the drug has influence. Then, replace the medium before adding CCK-8 Reagent to remove the influence of the drug.

6. Can CCK-8 Reagent be added to the experiment overnight and detected the next day?

In general, CCK-8 Reagent is recommended to be added and incubated at 37°C for 2 h before detection. If the time is too late, 1% SDS solution can be added to each well and stored in dark at room temperature. The absorbance value will not be affected within 24 h (the volume of 1% SDS solution is the same as that of CCK-8 Reagent).

7. If the measured absorbance is very low, how to solve it?

One is to increase the number of cells appropriately; the other is to extend the incubation time after adding CCK-8 Reagent.