CCK-8 Cell Counting Kit

A311

Version 22.1

Product Description

CCK-8 Cell Counting Kit is based on WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] that is rapidly absorbing, highly sensitive and widely used in detection of cell proliferation and cytotoxicity. The amount of formazan dye generated by dehydrogenases in living cells is directly proportional to the number of living cells. Measure the Optical Density at 450 nm, using a microplate reader. The measurements can be indirectly shows the number of living cells. CCK-8 Cell Counting Kit contains CCK-8 Solution, which is a ready-to-use reagent. CCK-8 Solution can be directly added to cell samples. After Incubating the mixed solution for a certain period of time, the assay can be performed without preparing various components in advance.

Components

Components	A311-01 500 rxns (10 μl/rxn)	A311-02 1,000 rxns (10 μl/rxn)
CCK-8 Solution	5 ml	10 ml

Storage

Store at 2 ~ 8℃ and protect from light. Adjust the shipping method according to the destination.

Applications

It is applicable for cell proliferation assay, cytotoxicity assay and drug screening, etc.

Self-prepared Materials

Multi-channel pipettes (100 - 200 μl, 10 μl), microplate reader (with a 450 nm filter), 96 well plate, CO_2 incubator.

Notes

For research use only. Not for use in diagnostic procedures.

1. CCK-8 Solution is pink solution, please protect from light.
2. For the initial experiment, it is recommended to explore the optimal number of cell seeding and the optimal incubation time of CCK-8 Solution (General incubation time is 1 - 4 h).
3. Be careful not to introduce bubbles to the wells.
4. The assay of this kit depends on the reaction catalyzed by dehydrogenase, so oxidizing and reducing agents will interfere with the assay. Please remove interfering substances before use and then assay with CCK-8 Solution.
5. If the drug contains metals (Pb^{2+}, Fe^{2+}, Cu^{2+}, etc.), it will interfere with the color reaction of CCK-8 Solution, resulting in the decrease of detection sensitivity.
6. When measuring cell number, in order to ensure the stability and repeatability of the test results, it recommended drawing a standard curve at the same time.

Experiment Process

1. Inoculate cells to a 96 well plate in a final volume of 100 μl;
   ▲ without drug treatment, skip directly to step 5 after step 1.
2. Pre-incubate in incubator (The incubation time depends on the requirements of specific experiments. The recommended time is 18 - 24 h.);
3. Add the corresponding drug to the well plate;
4. Incubate in a 37℃ cell culture incubator;
5. Add 10 μl CCK-8 Solution to a 96 well plate (if not a 96 well plate, make sure the CCK-8 Solution addition is 10% of the volume of medium per well in the cell plate);
6. Incubate in incubator for 1 - 4 h (time adjustable);
7. Use a microplate reader to detect absorbance values at 450 nm.

◇ **Standard Curve Drawing**

1. Collect and culture viable cells, calculate the number of cells in cell suspension with blood count plate, and then seed cells.
2. Inoculate cell suspension in a 96-well plate, dilute cells with medium in equal ratio gradient (such as 1:2). 5 - 7 cell concentration gradients are recommended, with 3 - 6 replications in each group. Add 100 µl of cell suspension to each well.
3. Add 10 µl of the CCK-8 Solution to each well of the plate, and mix gently. Incubate the plate in the 37℃ cell incubator for a period of time (Incubate for 1 - 4 h according to different cell types). Measure the absorbance at 450 nm using a microplate reader. Take cell number as the abscissa, absorbance values as the ordinate to draw standard curve. Cell number of unknown samples can be measured according to the standard curve. The precondition of using the standard curve is that the test conditions is exactly the same.

◇ **Cell activity assay**

1. Inoculate cell suspension (100 µl/well) in a 96-well plate. Pre-incubate the plate in a humidified incubator (e.g., at 37℃, 5% CO_2) for 24 h. Set the blank group and the control group at the same time.
2. Add 10 µl of the CCK-8 Solution to each well of the plate. (It is recommended to shake gently after adding along the cell wall to avoid bubbles).
3. Incubate the plate for 1 - 4 h in the incubator.
4. Measure the absorbance at 450 nm using a microplate reader.

◇ **Cell Proliferation and Cytotoxicity Assay**

1. Dispense 100 µl of cell suspension (100 µl/well) in a 96-well plate. Pre-incubate the plate for 24 h in a humidified incubator. Set the blank group and the control group at the same time.
2. Add different concentrations of substances to be tested to the plate. Incubate the plate for an appropriate length of time (Based on the drug being tested) in the incubator.
3. Add 10 µl of CCK-8 Solution to each well of the plate. (It is recommended to shake gently after adding along the cell wall to avoid bubbles) Incubate the plate in the incubator for an appropriate length of time.
4. Measure the absorbance at 450 nm using a microplate reader.

◇ **Reduction Formula**

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

Inhibition%=[(B-A)/(B-C)] × 100%

A: Experimental group OD (contain medium, cells, drugs and CCK-8 Solution)

B: Control OD (contain medium, cells, CCK-8 Solution)

C: Blank OD (contain medium, CCK-8 Solution)

FAQ and Troubleshooting

◇How many cells should there be in a 96 well?

At least 1,000 adherent cells/well (100 µl medium) and at least 2,500 leukocytes/well (100 µl medium). It is suggested that several holes with different cell numbers should be set before the experiment.

◇Is CCK-8 Solution toxic to cells?

CCK-8 Solution has low cytotoxicity and will not affect cell growth. The cells treated with CCK-8 Solution can discard the supernatant and then add cell culture medium to continue culture.

◇what are the factors affecting the measured value of CCK-8?

①Bubbles; ②The presence of oxidizing or reducing substances in the test system reduces and increases the test results accordingly.

◇How to operate when the drug to be tested is oxidizing or reducing?

If the drug to be tested is oxidizing or reducing, the fresh medium can be replaced before adding CCK-8 Solution to remove the effect of the drug to be tested. When the influence of the drug to be tested is relatively small, the blank absorption value after the drug to be tested is directly deducted without changing the culture medium.

◇Can CCK-8 Solution be added during the experiment and tested the next day after overnight?

In general, it is recommended to test after add CCK-8 Solution and incubate at 37℃ for 2 h. If the time is too late, 1% SDS solution can be added to each well, stored away from light at room temperature, and tested within 24 h, and the absorbance value will not be affected (the volume of 1% SDS solution is the same as that of CCK-8 Solution).

◇If the 96 well plate is not used to treat cells, how to determine the volume of CCK-8 Solution?

The addition amount of CCK-8 Solution is 10% of the volume of medium per well in the cell plate, which can be converted according to this proportion. If 384 well plate is used for cell proliferation and activity detection, it is recommended to dilute CCK-8 Solution with ddH_2O in the ratio of 1:1 and add 20% of the volume of culture medium per well in the cell plate.

◇How to solve the low measured absorbance value?

①Increase the number of cells; ②Extend the incubation time after adding CCK-8 Solution.