CCK-8 Cell Counting Kit

A311

Version 8.1

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Introduction

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. WST-8, similar to MTT, reduces to a water-soluble formazan dye in the presence of the electron carrying dehydrogenases found in mitochondria. 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 is a ready-to-use solution, can be directly added to cell supernatants, and incubated for a certain period of time and then test.

Components

Components	A311-01 500 rxn (10 μl/rxn)	A311-02 1000 rxn (10 μl/rxn)
CCK-8 Solution	5 ml	10 ml

Storage Conditions

Store CCK-8 solution at 4℃ protected from light.

Quality Control

Function test: Inoculate HEK293 cell suspension (100 μl/well) in a 96-well plate, set 3 repeat for one group and 9 gradient of cell numbers, cell number of each group is: 0、400、800、1600、3200、6400、12800、25600、51200/ well. Draw a standard curve on the absorbance value by the number of cells, the correlation $R^2 > 0.99$.

Notes

1. CCK-8 Cell Counting Kit is pink solution, please protect from light.
2. The incubation time varies by the type and number of cells in a well. Please set several parallel well to make sure the appropriate amount of cell and the incubate time of CCK-8 solution.(Generally set the incubate time from 1h to 4h) Be careful not to introduce 3.
3. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.
4. Since the CCK-8 assay is based on the dehydrogenase activity detection in viable cells, reducing agents (such as antioxidant) interfere with testing. Please try to remove reductant before using CCK-8 Solution.
5. In the experiments of pharmacological inhibition, if the metal is contained in drugs, such as Pb^{2+}, Fe^{2+}, Cu^{2+}, will effect on color reaction of CCK-8 Solution, leading to lower the sensitivity of detection.
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.

Required Equipment and Materials

100 - 200 μl multi-channel pipettes
plate reader (450 nm filter)
96-well plate
CO_2 incubator

Protocol

This kit can be used for cell proliferation induced by cytokines and cytotoxicity test caused anti-cancer drugs, and drug-induced cell growth inhibition test.

1. 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 (100 µl/well) in a 96-well plate, dilute cells with medium in equal ratio gradient (such as 1:2), 5 to 7 cell concentration gradients were generally performed, with 3 to 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. Incubate the plate in the incubator for an appropriate length of time. 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.

2. Cell Number Determination

1/ Inoculate cell suspension (100 µl/well) in a 96-well plate. Pre-incubate the plate in a humidified incubator (e.g., at 37°C, 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. (Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading).

3/ Incubate the plate for 1 - 4 hours in the incubator.

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

3. Cell Proliferation and Cytotoxicity Assay

1/ Dispense 100 µl of cell suspension (5000 cells/well) in a 96-well plate. Pre-incubate the plate for 24 hours in a humidified incubator (e.g., at 37°C, 5% CO_2). Set the blank group and the control group at the same time.

2/ Add 10 µl of various concentrations of substances to be tested to the plate. Incubate the plate for an appropriate length of time (e.g., 6, 12, 24 or 48 hours) in the incubator.

3/ 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 O.D. reading.) Incubate the plate in the incubator for an appropriate length of time.

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

4. 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)

FAQs and Troubleshooting

◇How many cells should there be in a well?

For adhesive cells, at least 1000 cells are necessary per well (100 µl medium). For leukocytes, at least 2500 cells are necessary per well (100 µl medium) because of low sensitivity. It is recommended to set several wells with different cell number to determine the condition.

◇Is CCK-8 toxic to cells?

Since the toxicity of CCK-8 is so low, the same cells can be used for other cell proliferation assays such as the crystal violet assay, neutral red assay or DNA fluorometric assay after the CCK-8 assay is completed.

◇When the drug are oxidizing or reducing, how to operate?

When the drug are oxidizing or reducing, you can replace the medium with fresh medium before adding CCK-8 Solution to remove the influence of drugs. When under test drug effect is small, you do not need to replace the medium, but directly deduce the blank absorption of the medium after adding the drug.

◇Could the test be performed the next day after adding CCK-8 solution?

In general, it is suggested to test when incubation at 37℃ for 2 h after adding CCK-8 solution. If not, please add 1% SDS and store at room temperature away from light. The absorbance won't be affected within 24 h (the volume of 1% SDS added is the same with that of CCK-8 solution).

◇How to determine the volume of CCK-8 Solution when you not use a 96-well plate?

The adding volume of CCK-8 Solution is 10% of the total volume of each well. If use 384-well plate, it is suggest to dilute the CCK-8 Solution 1 time using ddH_2O, and add 20% of the well volume of it.

◇If the measured absorbance value is low, how to solve?

①To increase the number of cells;

②Extend incubation time after adding CCK-8 Solution