CCK-8 Cell Proliferation and Cytotoxicity Assay Kit

Introduction:

Cell Proliferation and Cytotoxicity Assay Kit (Cell Counting Kit-8, CCK-8) are application of a new water soluble tetrazolium salt 2- (2- methoxy -4- - phenyl) -3- (4- - phenyl) -5- (2,4- two sulfophenyl) -2H- tetrazolium monosodium salt with high sensitivity for the detection of cell proliferation and cytotoxicity of colorimetric detection products.

In the presence of electron carriers,the tetrazolium salt can be tetrazolium into Formazan by some mitochondrial dehydrogenase. The Formazan is orange and water soluble, the generation of Formazan and cell viability is proportional. Cell proliferation more faster, more darker; cell toxicitym more bigger, the shallow. For the same cell, there is a linear relationship between the number of cells and the color depth.

The CCK-8 solution can be directly added into the cells, do not require pre equipped, the CCK-8 solution is very stable, no cell toxicitym, can incubation the cells for a long time. The method of CCK-8 is high sensitivity, no radioactivity,it is sensitive than other methods such as MTT, XTT, MTS and WST-1 in cell proliferation assay.

Product features:

- Convenient use: using only one single reagent;
- High sensitivity, reliable data, good repeatability, widely linear range;
- Results stable: the reagent is high stability, and experimental results reliable;
- No radioactive isotope and organic solvent, low toxicity to cells;
- Suitable for high-throughput drug screening.

Contents:

Cat No.: BD0079-1(500T)	Cat No.: BD0079-2(1000T)	Cat No.: BD0062-3(3000T)
CCK-8 Solution,5ml	CCK-8 Solution,10ml	CCK-8 Solution,30ml

Procedure:

Cell activity, Proliferation and Cytotoxicity Assay

1) Collection cells, and add cell suspension 100ul (5000-10000 cells) to 96-well microplates. (edge holes of the plate with sterile water or PBS filling). Each board control (add 100ul medium).

2) 37℃, 5% CO2 incubated overnight, observed under inverted microscope.

3) Each hole adding 10 ul of Assay Solution into each well. Mix the reagents by shaking the plate gently for 30 seconds. 37℃ incubation. (when the cell needs to treat with drug)

4) Each hole adding 10ul CCK-8 solution, 37℃ incubating for 1-4 hours.

5) Monitor the absorbance of the 450 nm value, if there is no 450nm filter, you can use the 450-490nm filter.

6) Set blank well at the same time (medium and CCK-8 solution, no cell), the control hole (a cell not

adding medium and CCK-8 solution, 3-5), each set of complex well.

PS: *If not determination of the OD value, we can add10 ul 0.1 M 1% HCl solution or w/v SDS solution to each well , and cover plate to avoid light preservation at room temperature, the value will not change in 24 hours.

*If the substance to be tested with oxidizing or reducing,it can be replaced with fresh medium before add CCK-8, to remove the effects of the drug. Of course, the medium can not replace if the drug case was small, deducted the culture medium of blank drug absorption directly.

7) Cell viability calculated:

Each test well OD value minus zero well OD or control well OD. Average the OD of repeat wells.

Cell viability% = (dosing cell OD- blank OD/ control cellsOD - blank OD)*100

Determination of cell number

1) First count the cell number use the cell count in the cell suspension, then inoculated cells.

2) Do a cell concentration gradient according to the proportion (for example: 1/2 ratio) followed by medium, generally do 3-5 cell concentration gradient, each 3-6 complex well.

3) Culturing 2-4 hours for the cells attached to the wall, and then add CCK-8 reagent after a certain period of time to determine the O.D value, making a number of cells as horizontal axis (X axis), the O.D value for the vertical axis (Y axis) of the standard curve. According to the standard curve can be determined the number of unknown samples(the experimental conditions should be consistent, easy to determine the number of cells inoculated and accession to the CCK-8 after the incubation time).

Attention：

1) When attention the cell suspension must be mixed to avoid cell settling down, We can mixing at times for each well has close cell numbers. In order to reduce errors, the circle holes of the culture plate can only add medium, rather than as a detection hole.

2) The culture time according to the cell types and the cell numbers. In general, the white cells are difficult to stain, so need to cultivate a long time or increase the number of cells (105 cells / well). Suspension cells compared with adherent cells to stain. The suspension cells can be removed from the incubator after 1-4 hours culture, observation or enzyme-labeled instrument determination. If the stain is difficult, the culture plate can be back into the incubator, culture a few hours and then determine. The best time to dyeing can be the best time of cultivation for suspended cells. For adherent cells, the culture time is generally 1- 4 hours, but the degree of staining can be observated by the naked eye for 30 minutes (depending on the cell type, need to try to find out the conditions).

3) A multi channel pipettors can reduce the difference between parallel well. When add CCK- 8 reagent, suggested ramp leading culture siding, do not plug into the culture medium, easy to have a bubble, which would interfere with O.D reading.

4) If the cell culture time was too long, medium color change or a pH change, proposed replacement of fresh medium plus CCK- 8 reagent. Medium containing phenol red can be used for the determination of the kit to do cell activity.

5) If not using a 96 well plate assay, CCK-8 solution dosage corresponding proportional increase.

Storage & Shelf life:

Store at 2-8℃and keep in dark place, quality guarantee period is one year.

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