Cell Counting Kit-8
Cat#: orb1566783 (User Manual)

Product name: Cell Counting Kit-8 (CCK-8), Enhanced

Catalog number：orb1566783

Size：100T/ 500T/ 10000T/ 1000T/ 5000T

Product information

Components	orb1566783 100T	orb1566783 500T	orb1566783 10000T	orb1566783 1000T	orb1566783 5000T
CCK-8 enhanced solution	1 ml	5 ml	10 ml×10	10 ml×1	5 ml×10
Manual	1	1	1	1	1

Product introduction

Cell Counting kit -8 (CCK -8) is a WST -8-based kit widely used for rapid and high-sensitivity detection of Cell proliferation and cytotoxicity.

The CCK-8 reagent containing WST-8(chemical name: 2-(2-methoxy-4-nitrophenyl) -3-(4-nitrophenyl) -5-(2,4-disulfonic benzene) -2 h-tetrazole monosodium salt) is a MTT-like compound, in the presence of an electron-coupled reagent, 1-Methoxy-5-methylphenylazine dimethyl sulfate (1-Methoxy PMS) , can be reduced by dehydrogenase in mitochondria to Formazan, a highly water-soluble orange-yellow Formazan product (see Figure 1) , producing Formazan in direct proportion to the number of living cells. Therefore, this property can be used directly for cell proliferation and toxicity analysis, the more cells proliferate faster, the darker the color, the greater the cytotoxicity, the lighter the color.

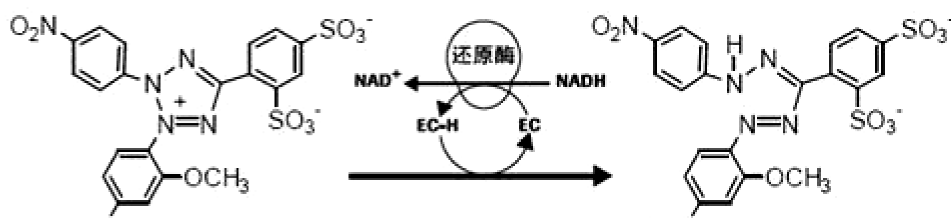

Figure 1. CCK-8 detection schematic diagram (EC=electron coupling reagent)

CCK-8 has obvious advantages over previous proliferation / toxicity assays (table 1) . Biorbyt CCK-8 is characterized by high sensitivity, short reaction time, wide linear range, reliable data and good repeatability, it can be widely used in drug screening, cell proliferation assay, cytotoxicity assay and tumor drug sensitivity test.

Table 1. Comparison of proliferation / toxicity assay reagents

Detection method	MTT	XTT	WST-1	CCK-8
Water-solubility of formazan	Poor (Organic solvents are required for dissolution)	Good	Good	Good
Detection sensitivity	High	Very high	Very high	Very high
Detection time	Long	Short	Short	Short
Detection wavelength	560-600nm	420-480nm	420-480nm	430-490nm
Cytotoxicity	High, cell morphology completely disappeared	Low, cell morphology remains unchanged	Low, cell morphology remains unchanged	Low, cell morphology remains unchanged
Reagent stability	General	Poor	General	Very good
Batch sample testing	Yes	Yes	Yes	Yes
Convenience	General	Good	Good	Very good

Operation Steps

Draw standard curve (when measure the particular cell amount)

1. Preparation of cell suspension: cell count.

2. Inoculating cells into 96-well plates: diluting to a cell concentration gradient in proportion (e. g. 1/2) with medium in equal proportion, generally 3-5 cell concentration gradients, 3-6 repeated wells in each group. 100 pl cell suspension per well.

3. Culture in 37 ° C incubator: it takes about 2-4 hours for cells to adhere to the wall after inoculation. If adherence is not required, this step can be omitted.

4. Add 10pl CCK-8-enhanced solution per well: since the amount of CCK-8 added per well is relatively small, it may be due to the reagent stuck on the hole wall, it is recommended to gently tap the plate after adding the reagent to help mix. Or directly prepared with 10% CCK -8 medium, in the form of fluid exchange. Be careful not to create bubbles in the wells, so as not to affect the detection of absorbance.

5. After incubation in the incubator for a certain period of time, the absorbance at 450 nm was measured, and a standard curve was made with the number of cells as the horizontal axis (X axis) and the absorbance as the vertical axis (Y axis) . According to this standard curve, the number of cells of unknown samples can be determined. (the standard curve is used on the premise that the experimental conditions should be consistent, so that the number of cells inoculated and the time of culture after adding CCK-8 can be determined.)

Detection of cell activity

1. Preparation of cell suspension: cell count.

2. Inoculate into 96-well plate: 100 pl cell suspension per well, 3 duplicate wells can be set according to the suitable number of plate cells.

3. Culture in 37 ° C incubator: it takes about 24 hours for cells to adhere to the wall after inoculation. If adherence is not required, this step can be omitted. Can also be based on the different requirements of the experiment, the corresponding time.

4. Add 10pl CCK-8-enhanced solution per well: since the amount of CCK-8 added per well is relatively small, it may be due to the reagent stuck on the hole wall, it is recommended to gently tap the plate after adding the reagent to help mix. Or directly prepared with 10% CCK -8 medium, in the form of fluid exchange. Be careful not to create bubbles in the pores, so as not to affect the detection of absorbance.

5. Incubation in the incubator for 0.5-4 hours: the amount of formazan formed varies for different cell types, and in most cases only 1 hour is required. If the color is not enough, the culture can be continued to confirm the best conditions for incubation. In particular, blood cells form less formazan, requiring longer color development time (5-6 hours) .

6. Determination of 450 nm absorbance: if the absorbance is not measured temporarily, 10 pl of CCK-8 reaction termination solution (Biorbyt, orb1566783) can be added to each well and stored at 2-8 ° C in a shaded culture plate, the absorbance does not change within 7 days; alternatively, 10 pl of self-prepared 0.1 m HCl solution or 1% w/v SDS solution

can be added to each well and covered, and the culture plate is kept away from light at room temperature, the absorbance does not change within 24 hours.

Detection of cell proliferation-toxicity

1. Prepare cell suspension: Cell count.

2. Inoculated into 96- well plates: Depending on the appropriate number of plated cells, 3 repeat wells can be set for each well of 100pl cell suspension.

3. Culture in 37 ° C incubator: it takes about 24 hours for cells to adhere to the wall after inoculation. If adherence is not required, this step can be omitted. Can also be based on the different requirements of the experiment, the corresponding time.

4. Add 0-10pl different concentrations of drugs which need to be tested to each well.

5. Culture in 37℃ incubator: The culture time of drugs added depends on the nature of the drugs and the sensitivity of the cells, which is usually determined by the cell cycle, at least for more than one generation.

6. Adding 10pl CCK-8-enhanced solution per well: Since the amount of CCK-8 added per well is small, it is possible to cause errors due to reagent sticking to the well wall, it is recommended to gently tap the culture plate after adding reagent to help mix. Alternatively, the medium containing 10% CCK-% was prepared directly and added as a replacement solution. Care should be taken not to generate bubbles in the holes to prevent detection of absorbance.

Note: If the drugs to be tested is oxidizing or reducing, remove the medium before adding CCK-8, wash the cells twice with the medium, and add a new medium to remove the effect of the drug. Of course, when the influence of drugs is relatively small, the blank absorption after adding drugs into the culture medium can be deducted directly without changing the culture medium.)

7. Incubation in the incubator for 0.5-4 hours: the amount of formazan formed varies for different cell types, and in most cases only 1 hour is required. If the color is not enough, the culture can be continued to confirm the best conditions for incubation. In particular, blood cells form less formazan, requiring longer color development time (5-6 hours) .

8. Determination of absorbance at 450 nm: It is recommended to use dual wavelengths, detection wavelength 450 - 490nm, reference wavelength 600 - 650nm. If the absorbance is not measured temporarily, 10 pl of CCK-8 reaction

termination solution (Biorbyt, orb1566783) can be added to each well and stored at 2-8 ° C in a shaded culture plate, the absorbance does not change within 7 days; alternatively, 10 pl of self-prepared 0.1 m HCl solution or 1% w/v SDS solution can be added to each well and covered, and the culture plate is kept away from light at room temperature, the absorbance does not change within 24 hours.

Calculation Formula

Cell survival rate = [(As - Ab) / (Ac - Ab)] ×100%

Inhibition rate = [(Ac - As) / (Ac - Ab)] ×100%

As ： The absorbance of the test wells (containing the cell's medium, CCK-8, and the drug)

Ac： The absorbance of the control wells(containing the cell's medium, CCK-8, no drug)

Ab ： The absorbance of the blank wells (No cell's medium, CCK-8)

Preservation Conditions

Protected from light at 4 ° C, valid for one year. -20 ° C can be stored longer, but repeated freezing and thawing will increase the background value and interfere with the assay, so keep the frequently used reagent at 4C.

Precautions

1. It is recommended to explore the number of cells inoculated with several wells and the incubation time after adding CCK-8 reagent.

2. Determination of CCK-8 reaction time: In general, leukocyte color development is difficult, so it is necessary to increase the number of cells and prolong the CCK-8 reaction time. The suspended cells are difficult to develop color compared with the adherent cells. Therefore, the suspended cells can be taken out from the incubator after being cultured for 0.5-4 hours with CCK-8, and the color development degree can be measured by visual inspection or microplate reader. If the color development is difficult, it can be determined after several hours. For adherent cells, the culture time of CCK-8 was generally 0.5-4 hours, and the color was observed by naked eyes after 20 min culture.

3. Number of cells per well: When using standard 96- well plates, the minimum amount of adherent cells is at least 1000 cells/well in 100 pl medium. The sensitivity of leukocyte detection is relatively low, so it is recommended to inoculate no less than 2500 cells/well in 100 pl medium. If a 24- or six-well plate experiment is to be used, calculate the amount of inoculum per well and add CCK-8 solution at 10% of the total volume of medium per well.

4. Set blank control: Add CCK-8 in the culture medium without cells, culture for a certain time, and measure the absorbance of 450 nm as blank control. In the experiment of cytotoxicity, the absorption of the drug should also be considered. CCK-8 can be added into the medium of the drug for a certain period of time, and the absorbance of 450 nm should be measured as the blank control.

5. Substances affecting the determination of CCK-8: Since the principle of detecting cell activity by CCK-8 is to detect the reaction catalyzed by dehydrogenase of living cells, if there is redox substance in the system to be tested, it may interfere with the detection result, the absorbance of the reductive substance will increase, and the absorbance of the oxidizing substance will decrease, so the effect of these substances should be removed by the method. Phenolic red and serum had no significant influence on the determination of testing kit, and the absorbance of phenolic red in culture medium could be eliminated by deducting the background absorbance in the control well containing only culture medium, so the test result would not be affected.

6. Determination wavelength: If the sample is a cell suspension with high turbidity, it is recommended to use dual wavelengths, detection wavelength 450 nm, reference wavelength 600 -650nm. Without a 450 nm filter, a filter with an absorbance between 430 - 490nm can be used, but with the highest detection sensitivity at 450 nm.

7. When cultured in the incubator, the holes in the outermost circle of the plate are most susceptible to drying and volatilization, increasing errors due to volume inaccuracy. In general, the outermost circle of holes is only added with culture medium and not used for measuring holes.

8.If the culture time is long and the color of the medium changes, the cells should be washed and changed before CCK-8 detection.

9. For your safety and health, wear a lab coat and disposable gloves.