ClearV Cell Counting Kit (CCK-8)

Product description

Cell Counting Kit-8, abbreviated as CCK-8, is a rapid and highly sensitive assay for cell proliferation and cytotoxicity based on WST-8 (chemical name: 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2, 4-disulfophenyl)-2H-tetrazolium monosodium salt), which is an upgraded version of MTT. WST-8 is an upgraded product of MTT, the working principle is: in the presence of electronically coupled reagents, it can be reduced by the dehydrogenase enzyme in the mitochondria to generate a highly water-soluble orange-yellow dirty product (formazan). The shade of color is directly proportional to cell proliferation and inversely proportional to cytotoxicity. The OD value is measured at 450 nm using an enzyme marker, indirectly reflecting the number of viable cells.CCK-8 method has a wide range of applications, such as drug screening, cell proliferation assay, cytotoxicity assay, tumor sensitivity assay, and the assessment of biological factor activity.

Components

Components No.	C331301E	C331301S	C331301M	C331301L	C331301T
Size	100 T	500 T	1000 T	3×1000 T	10×1000 T

Shipping and Storage

Ice packs shipping. 2~8°C dry and lightproof storage, valid for one year.-20°C dry and lightproof storage, valid for two years.

Notes

1. It is recommended to do a few wells first to feel the number of inoculated cells and the incubation time after adding CCK-8 reagent.
2. If conditions permit, it is suggested to use a multi-channel pipette to reduce differences between parallel wells. When adding the CCK-8 reagent, it is recommended to add it along the wall of the culture plate, rather than inserting it below the surface of the culture medium, as this may create bubbles that could interfere with OD readings.
3. Leukocytes may need to be cultured for a longer period of time.
4. When using a standard 96-well plate, the minimum inoculum of adherent cells should be at least 1000 cells/well (100 μL of medium). The sensitivity of leukocyte detection is relatively low, so an inoculum size of no less than 2500 cells/well (100 μL of medium) is recommended. For 24-well or 6-well plate experiments, please calculate the corresponding inoculum volume per well and add CCK-8 solution at 10% of the total volume of medium in each well.
5. If a 450 nm filter is not available, a filter with absorbance between 430~490 nm can be used,

but the 450 nm filter has the highest detection sensitivity.

6. The absorbance of phenol red in the medium can be eliminated by subtracting the absorbance of the background in the blank wells during the calculation, so it will not affect the assay.
7. For your safety and health, please wear a lab coat and disposable gloves.
8. For research use only.

Instructions

1. Cell activity assay

1) First count the number of cells in the prepared cell suspension with a cell counting plate, and then inoculate the cells into the culture plate.

2) Sequentially dilute the cells into a cell concentration gradient in proportion (e.g., 1/2 ratio) with culture medium isobologically, generally 3~5 cell concentration gradients should be made, and 3-6 replicate wells are recommended for each concentration.

3) After inoculation, incubate the cells for 2~4 h to make the cells stick to the wall, then add CCK-8 reagent to measure the OD value after incubation for a certain period of time, and make a standard curve with the number of cells as the horizontal coordinate (X-axis) and the OD value as the vertical coordinate (Y-axis). According to this standard curve, the number of cells in an unknown sample can be determined (the prerequisite for using this standard curve is that the conditions of the experiment should be consistent, so that it is easy to determine the number of inoculated cells and the incubation time after adding CCK-8).

2. Cell activity assay

1) Inoculate cell suspension (100 μL/well) in a 96-well plate. Place the plates in an incubator for a period of pre-culture (37°C, 5% CO_2).

2) Add 10 μL of CCK-8 solution to each well (be careful not to generate air bubbles in the wells, they will affect the OD reading).

3) Incubate the plate in the CO_2 incubator for 1~4 h.

4) Determine the absorbance at 450 nm using an enzyme meter.

5) If the OD value is not determined for the time being, 10 μL of 0.1 M HCL solution or 1% w/v SDS solution can be added to each well and the plates are covered and stored at room temperature away from light. the absorbance will not change when measured within 24 h. The absorbance will not change.

3. Cell proliferation-toxicity assay

1) Prepare 100 μL of cell suspension in a 96-well plate. Pre-culture the plate in an incubator for 24 h (37°C, 5% CO_2).

2) Add 10 μL of different concentrations of the substance to be tested to the culture plate.

3) Incubate the culture plate in the incubator for an appropriate period of time (e.g., 6, 12, 24 or 48 h).

4) Add 10 μL of CCK-8 solution to each well (be careful not to generate air bubbles in the wells again, they will affect the OD readings).

5) Incubate the plates in an incubator for 1~4 h.

6) Determine the absorbance at 450 nm using an enzyme meter.

7) If the OD value is not determined for the time being, 10 μL of 0.1 M HCL solution or 1% w/v SDS solution can be added to each well and the plates can be covered and stored at room temperature away from light. the absorbance will not change when measured within 24 h. The absorbance will not change when measured at room temperature.

【Note】If the test substance is oxidative or reductive, replace the medium with fresh medium before adding CCK-8 (remove the medium, wash the cells twice with medium, then add fresh medium) to remove the influence of the drug. Alternatively, if the influence of the drug is minimal, the medium can be not replaced, and the absorbance of the medium with the drug added can be subtracted directly from the blank absorbance.

4. Viability Calculation

Cell viability * (%) = [A (drugged) - A (blank)] / [A (0 drugged) - A (blank)]×100

A (dosing): absorbance of wells with cells, CCK-8 solution and drug solution

A (blank): absorbance of wells with medium and CCK-8 solution but no cells

A (0 dosing): absorbance of wells with cells, CCK-8 solution and no drug solution

*Cell viability: cell proliferation viability or cytotoxicity viability.