Cell Counting Kit - 8 (CCK - 8) Cell Viability Assay Kit

(Cat/No.:BC170 Size:100T)

1. Principle

The CCK - 8 cell viability assay kit contains WST - 8 [2-(2 - methoxy - 4 - nitrophenyl)-3-(4 - nitrophenyl)-5-(2,4 - disulfonylbenzene)-2H - tetrazolyl monosodium salt]. In the presence of electron carriers, intracellular dehydrogenases oxidize and reduce WST - 8, generating a water - soluble orange - yellow formazan dye that can dissolve in the tissue culture medium. The quantity of formazan produced is directly proportional to the number of viable cells. The sterile CCK - 8 solution can be added directly to the cell culture medium without the necessity of pre - mixing with other components. The CCK - 8 method represents a highly sensitive, non - radioactive colorimetric assay for quantifying the number of viable cells in cell proliferation or toxicity experiments, and it serves as a viable alternative to the traditional MTT method.

2. Composition (The kit is valid for 1 year)

Catalog Number	Components	Specification	Storage Conditions
BC170	CCK - 8 solution	1ml (100T)	4 °C, protected from light, valid for one year

3. Required Equipment and Materials

- Low - speed centrifuge
- Microplate reader with a 450nm wavelength
- Plate shaker
- Micropipette
- 96 - well culture plate
- CO_2 incubator

4. Experimental Procedures

(1) Dispense 100 µl of the cell suspension (containing approximately 1×10^4 cells) into each well of a 96 - well plate. Conduct pre - culture of the plate in an incubator maintained at 37 °C with 5% CO_2 for 24 hours.

(2) Add 100 µl of either complete culture medium or complete culture medium supplemented with test samples at various concentrations to each well of the culture plate.

(3) Continue culturing the culture plate in the incubator for an appropriate duration (e.g., 48 hours or 72 hours).

(4) Pipette 10 µl of CCK - 8 solution into each well, taking care to avoid the formation of bubbles within the wells.

(5) Incubate the culture plate in the incubator for a period ranging from 1 to 4 hours.

(6) Measure the absorbance at 450 nm using a microplate reader.

(7) In the event that immediate measurement of the OD value is not required and a subsequent measurement is planned, add 10µl of 0.1 M HCl solution or 1% (W/V) SDS solution to each well. Cover the culture plate and store it at room temperature in the dark. The absorbance will remain stable within a 24 - hour period.

5. Data Analysis and Result Interpretation

(1) Cell Survival Rate Calculation: Subtract the background OD value (measured in wells containing complete medium and CCK - 8 but no cells) from the OD value of each test well. Subsequently, calculate the mean ± standard deviation (SD) of the OD values obtained from replicate wells.

The cell survival rate is expressed as T/C%, where T represents the OD value of the drug - treated cells and C represents the OD value of the control cells.

Cell survival rate (%) = (OD of drug - treated cells / OD of control cells) × 100

(2) Calculation of Inhibitory Concentrations: Determine the drug concentration corresponding to T/C = 50% (IC50) or the drug concentration corresponding to T/C = 10% (IC90).

(3)

6. Precautions and Notes

(1) During cell inoculation, ensure thorough mixing of the cell suspension to prevent cell sedimentation, which may result in unequal cell numbers among wells. It is advisable to remix the suspension after inoculating several wells. The culture medium in the peripheral wells of the culture plate is prone to evaporation. To minimize experimental errors, it is recommended to fill the wells on the four sides of the culture plate with only culture medium or sterile PBS and exclude them from indicator detection.

(2) The optimal reaction time of CCK - 8 varies depending on specific color - development requirements. For initial experiments, it is recommended to prepare multiple wells to determine the optimal cell seeding density and the optimal incubation time following the addition of the CCK - 8 reagent. Generally, white blood cells are more difficult to stain, necessitating either an extended CCK - 8 reaction time or an increased number of cells (approximately 10^5 cells/well). Suspension cells pose greater challenges in staining compared to adherent cells. For suspension cells, after adding CCK - 8 and incubating for 1 to 4 hours, remove the plate from the incubator to visually assess the staining degree or use a microplate reader to determine the optimal incubation time. If staining is insufficient, return the culture plate to the incubator and continue culturing for additional hours before measurement. For adherent cells, the typical incubation time for CCK - 8 ranges from 1 to 4 hours. Most adherent cells can be visually

inspected after approximately 30 minutes of incubation, and optimal detection results are usually achieved after around 3.5 hours of incubation.

(3) The detection mechanism of this kit relies on the dehydrogenase - catalyzed reaction. Excessive reducing agents present in the test system, such as certain antioxidants, may interfere with the detection process and should be eliminated. The presence of phenol red in the culture medium does not affect the determination of cell viability using this kit.

(4) Assessment of Reducing Substances in the Test Solution: Add 10ul of CCK - 8 solution to a well containing only the test solution (without cells), incubate for 1 to 4 hours, and measure the blank absorbance at 450nm. A very low absorbance value indicates the presence of minimal reducing agents in the test system, allowing for the direct addition of CCK - 8 during the formal test. Conversely, a relatively high absorbance value suggests the presence of significant reducing agents. In such cases, for the formal test, remove the culture medium, wash the cells

(5) When dealing with cell suspensions of high turbidity, it is recommended to set 600 nm (or a wavelength above 600 nm) as the reference wavelength and subtract the OD value measured at this reference wavelength.

(6) Appropriate personal protective equipment, including a lab coat and disposable gloves, should be worn during the experiment.