

Cell Counting Kit-8 (CCK-8)

Introduction

Cell Counting Kit-8 (CCK-8) is a reagent used for cell proliferation/toxicity detection. It uses a highly water-soluble tetrazolium salt, WST-8, which is reduced in the presence of electron-coupled reagents by the abundant dehydrogenases in living cells to the formazan form, an orange dye that is soluble in culture media. The amount of formazan is directly proportional to the number of viable cells.

Compared with other common tetrazolium salts, such as MTT, MTS, XTT and WST-1, CK-8 has higher sensitivity and stability.

Storage

Store at -20°C away from light, stable for 1 year. For frequent use, store at 4°C away from light, stable for at least 3 months.

Protocol

1. Cell viability assay

- 1) Seed cells (100 μ L/well) in 96-well plates.
- 2) Add 10 μ L of CCK-8 to each well.

***Note:** Be careful not to introduce bubbles into the wells, as they can interfere with detection.

- 3) Incubate the plate in an incubator for 1-4 h.

***Note:** The incubation time can be adjusted according to the specific experiment.

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

***Note:** If absorbance is not measured immediately, add 10 μ L of 1% w/v SDS or 0.1 M HCl to each well, cover and store at room temperature protected from light. No change in absorbance is observed for 24 h.

2. Cell proliferation/toxicity assay

- 1) Seed 100 μ L of cell suspension (approximately 5000 cells/well) per well in 96-well plates. Incubate in a cell culture incubator for 24 h.
- 2) Add 10 μ L of different concentrations of the compounds to the well.

- 3) Incubate the plate in the incubator for an appropriate amount of time (e.g., 6, 12, 24, or 48 h) according to the experimental design.
- 4) Add 10 μ L of CCK-8 to each well.

***Note:** Be careful not to introduce bubbles into the wells, as they can interfere with detection.

- 5) Place the plate in the incubator and incubate for 1-4 h.

***Note:** The incubation time can be adjusted according to the specific experiment.

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

***Note:** If absorbance is not measured immediately, add 10 μ L of 1% w/v SDS or 0.1 M HCl to each well, cover and store at room temperature protected from light. No change in absorbance is observed for 24 h.

3. Data analysis

Cell viability (%) = $[(As-Ab) / (Ac-Ab)] \times 100\%$

Inhibition rate (%) = $[(Ac-As) / (Ac-Ab)] \times 100\%$

As = Absorbance of experiment group (absorbance of wells containing cells, medium, CCK-8, and tested compound)

Ab = Absorbance of blank group (absorbance of wells containing medium and CCK-8)

Ac = Absorbance of control group (absorbance of wells containing cells, medium, and CCK-8)

4. Standard curve (optional)

- 1) After cell counting, use cell medium to dilute the cell suspension in a series of concentration gradients, typically requiring 5-7 concentration gradients of three replicates per set. Seed cells in the 96-well plates as usual.

***Note:** pay attention to the number of cells per well. If the cell suspension is diluted in a tube, carefully mix the cells again before adding them to the plate. The volume of cell suspension in each well should be consistent.

- 2) Incubate until cells are adherent (typically 2-4 h), then add 10 μ L of CCK-8 per 100 μ L of medium. Continue incubating for 1-4 h and measure the absorbance at 450 nm with a microplate reader. Make a standard curve with the number of cells as the X-axis coordinate and the OD value as the Y-axis coordinate. Based on this curve, the number of cells in the sample to be tested can be determined. The prerequisite for using this standard curve is that the culture conditions are the same.

Note

1. For adhesive cells, at least 1000 cells per well (100 μ L medium) are necessary. For leukocytes, at least 2500 cells are necessary per well (100 μ L medium) on account of low sensitivity. The recommended maximum number of cells per well for the 96-well plate is 25000. If a 24-well or 6-well plate is used for this assay, please

calculate the number of cells per well correspondingly, and adjust the volume of the CCK-8 added to 10% of the total volume.

2. WST-8 might react with reducing agents to generate WST-8 formazan. Please check the background O.D. if reducing agents are used in cytotoxicity assays or cell proliferation assays.
3. Care about not introducing bubbles to the wells, since they interfere with the O.D. reading.
4. Measure and subtract the O.D. at 600 nm or higher from that of the sample if there is high turbidity in the cell suspension.
5. The absorption value of phenol red in a culture medium can be removed by subtracting the absorption value of a blank solution from the absorption value of each well. Therefore, a medium containing phenol red is usable for the CCK-8 assay.
6. The toxicity of CCK-8 is very low, the same cells might 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. (Don't recommend.)
7. This kit can be utilized for E.coli, but not yeast cells.
8. We recommend inoculating the cells near the center of the plate, culture medium in the most peripheral wells of the plate is easy to evaporate, you can fill these wells with PBS or water.
9. The addition of a metal ion in the reagent may affect CCK-8 sensitivity.
10. For your safety and health, please wear lab coats and gloves during the experiment.
11. For research use only. Not to be used in clinical diagnostic or clinical trials.

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