# Cell Counting Kit-8 (CCK-8) Plus

## Introduction

The Cell Counting Kit-8 (CCK-8) Plus is a convenient and sensitive cell proliferation/toxicity assay. 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 produced by dehydrogenase is directly and linearly related to the number of viable cells.

This product is optimized for detection with only a 0.5-1 hour incubation, which is more sensitive, faster, and has a wider linear range than the common CCK-8. At the same time, compared with other common tetrazolium salts, such as MTT, MTS, XTT and WST-1, this product also has higher sensitivity and stability.

## Components and Storage

| Components | K2268-100 T | K2268-500 T | K2268-1000 T | K2268-3000 T | K2268-10000 T |
|---|---|---|---|---|---|
| **Cell Counting Kit-8 (CCK-8) Plus** | 1×1 mL | 5×1 mL | 2×5 mL | 6×5 mL | 20×5 mL |
| Store at -20°C away from light, stable for 1 year. For frequent use, keep it at 4°C away from light, and stable for at least 2 weeks. | | | | | |

## Protocol

### 1. Cell viability assays

1) Cell suspensions (100 μL/well) are seeded in 96-well plates. Incubate in a cell culture incubator for a period.

2) Add 10 μL of CCK-8 Plus to each well.

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

3) Place the plate in the incubator and incubate for 0.5-3 h.

***Note:** For most cases, incubation for 0.5-1 h is sufficient. The incubation time can be adjusted according to the specific experiment.

4) Shake the plate on a shaker for a few seconds. Subsequently, measure the absorbance at 450 nm using a microplate reader.

***Note:** If absorbance is not measured immediately, add 10 μL 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 assays

1) Seed 100 µL 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 µL of different concentrations of the compound 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 µL of CCK-8 Plus 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 0.5-3 h.

***Note:** For most cases, incubation for 0.5-1 h is sufficient. The incubation time can be adjusted according to the specific experiment.

6) Shake the plate on a shaker for a few seconds. Subsequently, measure the absorbance at 450 nm using a microplate reader.

***Note:** If absorbance is not measured immediately, add 10 µL 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) ] X 100%

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

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

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

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

## 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 µL of CCK-8 Plus per 100 µL of medium. Continue incubating for 0.5-3 h and measure the absorbance at 450 nm with a microplate reader. A standard curve with the number of cells as the X-axis coordinate and the OD value as the Y-axis coordinate was made. 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 assay conditions are the same.

## Note

1. Repeated freeze-thaw of this product will affect its detection effect. Although repeated freeze-thaw 3 times will not significantly affect the detection effect, it is recommended to avoid repeated freeze-thaw as much as possible for better use effect. A small amount of precipitate may occur after freezing and thawing, and it can be equilibrated to room temperature and dissolved as much as possible. For short-term frequent use, store the product at 4°C and try to use it within 2 weeks.
2. If you want to use CCK-8 that can be stored at 4°C for a longer period, you can choose the Cell Counting Kit-8 (CCK-8) (Cat. No. K1018).
3. For adhesive cells, at least 1000 cells per well (100 μL medium) are necessary. For leukocytes, at least 2500 cells are necessary per well (100 μL 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 Plus added to 10% of the total volume.
4. Because the CCK-8 assay is based on the dehydrogenase activity detection in viable cells, conditions or chemicals that affect dehydrogenase activity may cause discrepancies between the actual viable cell number and the cell number determined using the CCK-8 assay.
5. 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.
6. Care about not introducing bubbles to the wells, since they interfere with the O.D. reading.
7. If you want to sterilize the CCK-8 solution, please filter the solution with a 0.2 μm membrane.
8. 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.
9. 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.
10. 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.)
11. This kit can be utilized for E.coli, but not yeast cells.
12. Before reading the plate, you can mix gently on an orbital shaker for homogenization.
13. 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.

14. If you don't have a 450 nm filter. You can use filters with an absorbance between 430 and 490 nm, even though a 450 nm filter gives the best sensitivity. Measure the absorbance at 450 nm and you can take the absorbance at 650 nm as a reference.

15. The addition of a metal ion in the reagent may affect CCK-8 sensitivity.

16. For your safety and health, please wear lab coats and gloves during the experiment.

17. For research use only. Not to be used in clinical diagnostic or clinical trials.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com