

# Cell Counting Kit-8 (CCK-8)

CAT NO.: APL-8C02

## DESCRIPTION

Cell Counting Kit-8 (CCK-8) allows convenient assays using WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt), which produces a water-soluble formazan dye upon bioreduction in the presence of an electron carrier, 1-Methoxy PMS. CCK-8 solution is added directly to the cells, no pre-mixing of components is required. WST-8 is bioreduced by cellular dehydrogenases to an orange formazan product that is soluble in tissue culture medium. The amount of formazan produced is directly proportional to the number of living cells. Since the CCK-8 solution is very stable and it has little cytotoxicity, a longer incubation, such as 24 to 48 hours, is possible.

Cell Counting Kit-8 allows sensitive colorimetric assays for the determination of the number of viable cells in the proliferation and cytotoxicity assays. The detection sensitivity is higher than any other tetrazolium salts such as MTT, XTT or MTS.

## CONTENT

APL-8C02	
Cell Counting Kit-8 (CCK-8)	5 mL

## STORAGE

Stored at 4°C protecting from light, and is stable for up to 12 months. Stored at -20°C protecting from light, and is stable for up to 2 years.

## APPLICATION

1. Cell proliferation determinations: Cell Counting Kit-8 (CCK-8) is water soluble, stable in culture, and non-toxic.
2. Cell viability assays: metabolic activity and dye generation changes in proportion to altered viability.
3. Cytokine assays-measure cytokine-induced proliferation. Cells can be recovered and expanded at the end of the study if desired.
4. Cytotoxicity assays-Cells death from cytotoxic chemicals has no effects on color development, only living cells convert the reagent into a colorimetric indicator. The reagent itself has negligible toxicity, and is generally safe for cells.

## PROTOCOL

### Cell Number Determination

1. Inoculate cell suspension (100  $\mu$ L/well) in a 96-well plate. Pre-incubate the plate in a humidified incubator (e.g., at 37°C, 5% CO<sub>2</sub>).
2. Add 10  $\mu$ L of the CCK8 solution to each well of the plate. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.

3. Incubate the plate for 1 - 4 hours in the incubator.
4. Measure the absorbance at 450 nm using a microplate reader.

#### Cell Proliferation and Cytotoxicity Assay

1. Seed cells in a 96-well plate at a density of 104-105 cells/well in 100  $\mu$ L of culture medium with or without compounds to be tested. Culture the cells in a CO<sub>2</sub> incubator at 37°C for 24 hours.
2. Add 10  $\mu$ L of various concentrations of substances to be tested to the plate.
3. Incubate the plate for an appropriate length of time (e.g., 6, 12, 24 or 48 hours) in the incubator.
4. Add 10  $\mu$ L of CCK8 solution to each well of the plate using a repeating pipettor. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.
5. Incubate the plate for 1 - 4 hours in the incubator.
6. Before reading the plate, it is important to mix gently on an orbital shaker for 1 minute to ensure homogeneous distribution of color.
7. Measure the absorbance at 450 nm using a microplate reader.

#### Data Analysis

There are several ways to do statistical analysis. You can choose to use O.D. values or cell numbers. We offer one of them.

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

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

As = absorbance of the experimental well (absorbance of cells, medium, CCK8 and wells of the test compound).

Ab = blank well absorbance (absorbance of wells containing medium and CCK8).

Ac = control well absorbance (absorbance of wells containing cells, medium and CCK8).

#### Making a standard curve

1. The cell counting plate counts the number of cells in the cell suspension.
2. Using the medium, the cell suspension is diluted to a concentration gradient, usually requiring 5-7 concentration gradients, several replicate wells per group. Then inoculate the cells. (Note the number of cells per well. If you are diluting the cell suspension in a tube, carefully mix the cells again before adding the wells to the plate. The volume of the cell suspension in each well should be the same.)
3. Incubate until the cells are adherent (usually 2-4 hours), then add 10  $\mu$ l of CCK8 per 100  $\mu$ l of medium. Incubation was continued for 1-4 hours, and the absorbance at 450 nm was measured with a microplate reader. Make a standard curve with the number of cells as the X-axis coordinate and the O.D. value as the Y-axis coordinate.
4. The number of cells of the sample to be tested can be determined based on the curve. A prerequisite for using this standard curve is that the culture conditions are the same.

#### Precautions

1. Make sure the drug and CCK8 are evenly distributed in the medium.
2. The more cells proliferate, the darker the color; the stronger the cytotoxicity, the lighter the color.
3. For adherent cells, at least 1000 cells per well (100  $\mu$ l medium). For leukocytes, at least 2500 cells per well (100  $\mu$ l medium) are required due to their low sensitivity. The recommended 96-well plate has a maximum cell count of

- 25,000 per well. If the test is performed using a 24-well or 6-well plate, calculate the corresponding number of cells per well and adjust the volume of CCK8 to 10% of the total liquid volume per well.
4. Since the CCK8 assay is based on dehydrogenase activity in living cells, conditions or chemicals that affect dehydrogenase activity may result in a difference between the actual number of viable cells and the number of viable cells measured using CCK8.
  5. WST-8 may react with a reducing agent to form WST-8 formazan. If a reducing agent (such as some antioxidants) is used, it will interfere with the test. If more reducing agent is present in the system to be tested, it is necessary to remove it.
  6. After 2 hours of incubation, the background O.D. value is typically 0.1-0.2 units.
  7. Be careful not to introduce air bubbles into the holes as they will interfere with the O.D. value.
  8. If you want to sterilize the CCK8 solution, use a 0.2  $\mu\text{m}$  membrane filter solution.
  9. The incubation time will vary depending on the type and amount of cells in the well. Generally, leukocytes are less colored and may require longer incubation times (up to 4 hours) or large numbers of cells (~105 cells/well).
  10. If there is high turbidity in the cell suspension, measure and subtract the O.D value of the sample at 600 nm or higher.
  11. CCK8 cannot be used for cell staining.
  12. The phenol red in the medium does not affect the experimental results. The absorbance of phenol red can be eliminated by subtracting the absorbance of the background in the blank hole during calculation, so it will not affect the detection.
  13. The toxicity of CCK8 is very low. After the CCK8 assay is completed, the same cells can be used for other cell proliferation assays, such as crystal violet assay, neutral red assay or DNA fluorescence assay. (Unless the cells are extremely rare, it is not recommended.)
  14. This kit can be used in E. coli but not in yeast cells.
  15. Before reading the plate, you can mix gently on the shaker.
  16. We recommend inoculation of cells in wells near the center of the plate. The medium in the outermost circle of holes is easily evaporated and can be filled with PBS, water or medium.
  17. If you don't have a 450 nm filter. You can also use filters with absorbance between 430 and 490 nm, and 450 nm filters for optimum sensitivity.
  18. Measure the absorbance at 450 nm. If you need to make a dual wavelength measurement, the absorbance at 650 nm can be determined as the reference wavelength.
  19. The presence of metal ions in the drug may affect the sensitivity of CCK8. Lead chloride, iron chloride, and copper sulfate at a final concentration of 1 mM inhibit 5%, 15%, and 90% of the color reaction, reducing sensitivity. If the final concentration is 10 mM, it will be 100% inhibited.

## PRODUCT USE LIMITATION

For Research Use Only. Not for Use in Humans.