

Product Data Sheet

Product Name: Cell Counting Kit-8 (CCK-8)

Cat. No.: GK10001

Features

Applications	1) Cell proliferation determinations-the GlpBio 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.
Shipping	Ship with blue ice.
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.
Usage	For Research Use Only! Not For Use in Humans.

Protocol

Cell Number Determination

1. Inoculate cell suspension (100 µL/well) in a 96-well plate. Pre-incubate the plate in a humidified incubator (e.g., at 37°C, 5% CO₂).
2. Add 10 µL 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 10^3 - 10^4 cells/well in 100 µL of culture medium to be tested. Culture the cells in a CO₂ incubator at 37°C for 24 hours.
2. Add 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 µL 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

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

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 µl of CCK8 per 100 µl 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.

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 µl medium). For leukocytes, at least 2500 cells per well (100 µl 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.

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

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 μm 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 ($\sim 10^5$ 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.

Frequently Asked Question

Q: How to conduct a blank control?

A: A blank control can be set up by adding the corresponding amount of cell culture medium and

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

CCK-8 but no cells. If there is concern that the drug being used might interfere with the detection, a blank control can be set up by adding the corresponding amount of cell culture medium, the drug, and CCK-8 but no cells.

Q: In the blank well that only contains CCK-8 but no cells, does the value increase?

A: Throughout the entire experiment, as time increases, if the cell culture time is long, please pay attention to the evaporation issue. Because the reaction environment temperature is high, some liquid will evaporate. Although the blank group has no cells, as the liquid evaporates, the drug concentration is equivalent to increasing. Therefore, the value of the blank control will also have a slight change (but there will not be a significant change). If you are very concerned about the value of the blank control, you can add culture medium, water or PBS around the 96-well plate to keep it moist. At the same time, you can place the 96-well plate in the incubator near the water tray to alleviate evaporation. Cover the outer two circles of the 96-well plate with PBS to prevent the evaporation from starting from the periphery, so as to minimize the impact on the interior and the blank control.

Q: How often is the value usually measured?

A: During the pre-experiment, the absorbance can be detected with a microplate reader at 0.5, 1, 2 and 4 hours respectively, and then a time point with a relatively suitable absorbance range can be selected for the subsequent experiment.

Q: After adding CCK-8, we don't measure the OD value for the time being. How can we handle it temporarily? How long can it be stored?

A: If you need to temporarily not measure the OD value, you can add 10ul of 0.1M HCl solution or 1% w/v SDS solution to each well, store it in the dark at room temperature, and the absorbance will not change within 24 hours.

Q: How to store?

A: The CCK-8 solution can be stored for 1 year under light-protected and 0-5°C conditions. If not used for a long time, it can be stored at -20°C while protected from light for 2 years. It is recommended to aliquot and store at -20°C. When using, thaw at 4°C in advance. Please avoid repeated freezing and thawing as it will increase the background value and interfere with the experimental results.

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

Q: Is it necessary to replace the culture medium?

A: No, if the impact of the drug is relatively small, there is no need to replace the culture medium. Just subtract the blank absorption after adding the drug to the culture medium.

Q: What should be done if the substance to be tested is oxidizing or reducing?

A: The detection of this kit relies on the reaction catalyzed by dehydrogenase. If the substance to be tested is oxidizing or reducing, you can replace the fresh culture medium before adding CCK-8, thereby eliminating the influence of the drug.

If there is a reducing agent in the experiment, please check the background OD value, that is, add the drug to the culture medium without cells, then add the CCK-8 reagent and detect within a certain period of time, and compare it with the culture medium without adding the drug (only with CCK-8 reagent). If the OD value is significantly higher, it indicates a reaction.

Q: If the color or pH of the culture medium has changed, is it necessary to replace it with a new one?

A: When adding CCK-8, if the cell culture time is long and the color or pH value of the culture medium has changed, it is recommended to use fresh culture medium.

Q: Which cells are applicable?

A: Since we are measuring mitochondrial enzymes, any eukaryotic cells with mitochondria are suitable, such as single-celled organisms and animal cells. However, cells with cell walls, such as plant cells and yeast cells, have a low efficiency for substrate entry into the cells, and therefore are not recommended.

Q: What are the reasons for poor linearity?

A: Uneven cells; evaporation of the culture medium; reducing agent; bubbles; the area around the well plate cannot be used unless the culture time is less than one day; the number of cells is very important; too few cells will result in a low signal.

Q: Metal toxicity?

A: Metal toxicity can be detected. It is best to conduct a blank control.

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

Q: Which cells are applicable? Are there any other cells that can be used? Bacterial cells within biofilms (such as *Haemophilus influenzae* and *Pseudomonas aeruginosa*)?

A: All mammalian cells. Bacteria generally do not have the ability to detect CCK-8. They can be detected by staining methods, or flow cytometry, the most common method. There have been reports that turbidimetry can be used to measure OD600.

Q: Can CCK-8 be used as an alternative to Brud or EDU methods for detecting cell proliferation?

A: Although CCK-8 measures the activity of cells in the growth phase by detecting dehydrogenase within the cells, and the thymidine incorporation method detects cell activity by inserting nucleotide analogues into the synthesized DNA, these methods are somewhat related. CCK-8 can replace the thymidine incorporation method for detecting cell proliferation, and the detection process is more convenient.

Q: The blank well values are too high. Could it be that the medium has become contaminated? Or is there some other reason?

A: Generally, high values in the blank wells are attributed to contamination. Such issues are rarely encountered.

Q: Can CCK8 detect tissues?

A: It cannot detect tissues.

Q: Can T cells be detected?

A: Yes, T cells grow slowly, so the incubation time needs to be extended.

Q: after a certain period of time, only the growth and proliferation of the bone marrow mesenchymal stem cells are measured. Can the CCK-8 assay be used for this purpose? How to do it?

A: Regarding the situation of the two types of cells:

(1) If the growth status of the adherent cells is to be measured, then the suspended T cells can be removed and the above second method can be used for measurement.

(2) If the T cells are crucial for the growth of the mesenchymal cells and cannot be removed, then the

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

measured activity level is the total amount. At this time, the T cells in each well need to be estimated. If the total amount of T cells under each culture condition is close, or if the content of T cells is very small and much less than that of the mesenchymal cells, it can also be measured. Using the control well can remove the influence of T cells.

(3) If the T cells can be removed within a short period of time, a sensitive CCK-8 can be selected with strong signal. It is recommended that the cell quantity is appropriate (10000 cells in each well), and the reading results can be obtained within 30 minutes after measurement. After measurement, the T cells can be re-added to the culture well. This will have little impact on the growth of the cells. However, if a kinetics study is conducted, more factors need to be considered.

Q: CCK-8 measures the number of cells by reacting with the dehydrogenase in the cells. If the cells have died but the dehydrogenase activity is still present, how can the number of cells be calculated?

A: In most cases, during the process of cell death, the components within the cells will degrade, and some dehydrogenase will be released. However, its activity is relatively low compared to that of living cells. For example: 20 hours after 100 cells die, the released dehydrogenase activity is less than that of 5 living cells. That means the error in this situation is within 5%. Moreover, for the same type of cells, the situation is the same. Generally, the same type of cells are compared between different wells, so the systematic error is the same, that is, the systematic error will cancel each other out.

Background

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 reduced by cellular dehydrogenases to an orange formazan product that is soluble in a tissue culture medium. The CCK-8 assay measures cell viability based on formazan production, which is directly proportional to the number of living cells. Since the CCK-8 solution is very stable and has little cytotoxicity, a longer incubation, such as 24 to 48 hours, is possible.

Cell Counting Kit-8 (CCK-8) allows sensitive colorimetric assays for the determination of the number of viable cells in the proliferation and cytotoxicity assays. The detection sensitivity of CCK-8 is superior to that of other tetrazolium salts, including MTT, XTT, and MTS.

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

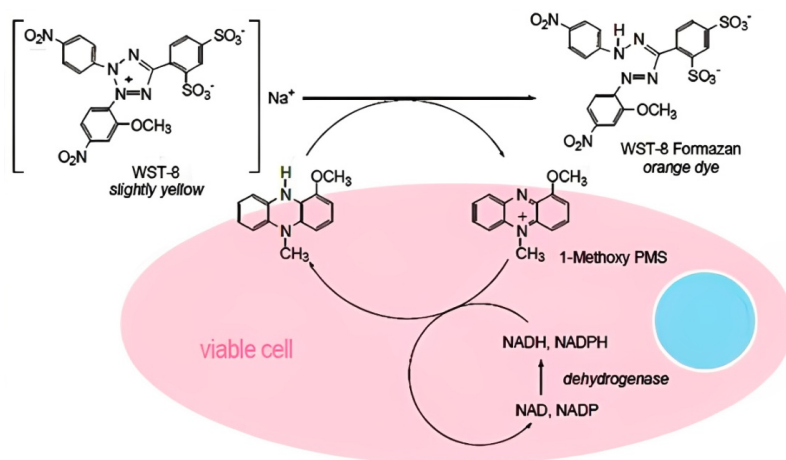


Figure 1: Working mechanisms of Cell Counting Kit-8 (CCK-8).

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA