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# Cell Counting Kit-8 (CCK-8)

## Catalog Number: A014-1, A014-2

## Introduction

The Cell Counting Kit-8 (CCK-8) is a colorimetric assay kit used to measure cell proliferation and cytotoxicity. CCK-8 utilizes a water-soluble tetrazolium salt WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] produces a water-soluble formazan dye upon reduction in the presence of an electron carrier, as shown in Figure 1. CCK-8 is a ready-to-use solution that does not require radioisotopes and correlates with the [$^3$H]-thymidine incorporation assay. CCK-8 can be added directly to the cell media for fast, high-throughput screening without a solubilization process obtaining highly reproducible and accurate results. CCK-8 allows sensitive colorimetric assays for the determination of the number of viable cells in cell proliferation and cytotoxicity assays. WST-8 is reduced by dehydrogenases in cells to give a yellow colored product (formazan), which is soluble in the tissue culture medium. The amount of the formazan dye generated by the activity of dehydrogenases in cells is directly proportional to the number of living cells. The detection sensitivity of CCK-8 is higher than other tetrazolium salts such as MTT, XTT, MTS or WST-1.

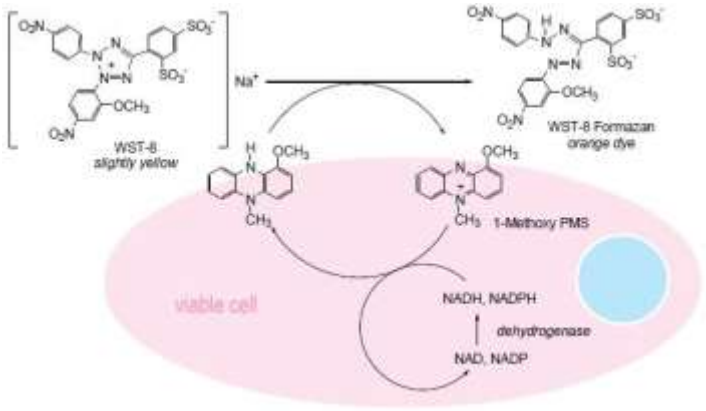


Figure 1. Principle of the cell viability assay using CCK-8

## Experimental Protocols

### Cell Proliferation Assay

1. Inoculate cell suspension (100 µL/well) in a 96-well plate. Also prepare wells that contain known numbers of viable cells (to create a calibration curve in step 5). Pre-incubate the plate in a humidified incubator (e.g., at 37°C, 5% $CO_2$).
2. Thaw the CCK-8 on the bench top or in a water bath at 37°C if it is frozen. It takes about 30 minutes on the bench top at 25°C or 5 minutes in a water bath at 37°C.
3. Add 10 µL of the CCK-8 solution to each well of the plate. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.
4. Incubate the plate for 1-4 hours in the incubator.
5. Measure the absorbance at 450 nm using a microplate reader. Prepare a calibration curve using the data obtained from the wells that contain known numbers of viable cells. To measure the absorbance later, add 10 µL of 1% w/v SDS to each well, cover the plate and store it with protection from light at

room temperature. No absorbance change should be observed for 48 hours.

## Cytotoxicity Assay

1. Dispense 100 µL of cell suspension (5000 cells/ well) in a 96-well plate.
2. Pre-incubate the plate for 24 hours in a humidified incubator (e.g., at 37 °C, 5% $CO_2$).
3. Add 10 µL of various concentrations of toxicant into the culture media in the plate.
4. Incubate the plate for an appropriate length of time (e.g., 6, 12, 24 or 48 hours) in the incubator.
5. Thaw the CCK-8 on the bench top or in a water bath at 37 °C if it is frozen. It takes about 30 minutes on the bench top at 25 °C or 5 minutes in the water bath at 37 °C.
6. Add 10 µL of CCK-8 solution to each well of the plate. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.
7. Incubate the plate for 1-4 hours in the incubator. Measure the absorbance at 450nm using a microplate reader. To measure the absorbance later, add 10 µL of 1% w/v SDS to each well, cover the plate and store it with protection from light at room temperature. No absorbance change should be observed for 48 hours.

**Notes:**

1. Since the CCK-8 assay is based on the dehydrogenase activity detection in viable cells, conditions or chemicals that affect dehydrogenase activity in viable cells may cause discrepancy between the actual viable cell number and the cell number determined using the CCK-8 assay.
2. WST-8 may 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. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.
4. Membrane filtration is recommended for the sterilization of the CCK-8 solution, if necessary
5. The incubation time varies by the type and number of cells in a well. Generally, leukocytes give weak coloration, thus a long incubation time (up to 4 hours) or a large number of cells (~$10^5$ cells/well) may be necessary.
6. Measure the reference wavelength at 600 nm or higher if there is a high turbidity in the cell suspension.

**FAQ:**

1. **How many cells should there be in a well?** For adhesive cells, at least 1000 cells are necessary per well (100 µL medium) when using the kit's standard 96-well plate. For leukocytes, at least 2500 cells are necessary per well (100 µL medium) because 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 accordingly, and adjust the volume of the CCK-8 solution in a well to 10% of the total volume.
2. **Does CCK-8 stain viable cells?** No, it does not stain viable cells because the water-soluble tetrazolium salt (WST-8) is used in the CCK-8 solution. The electron mediator, 1-Methoxy PMS, receives electrons from a viable cell and transfers the electron to WST-8 in the culture medium. Since its formazan dye is also highly water-soluble, CCK-8 cannot be utilized for cell staining purpose.
3. **Does phenol red affect the assay?** No. 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 phenol red containing medium is usable for the CCK-8 assay.
4. **Is CCK-8 toxic to cells?** The toxicity of CCK-8 is so low that, after the CCK-8 assay is completed, the same cells can be used for other cell proliferation assays such as the crystal violet assay, neutral red assay or DNA fluorometric assay.
5. **I do not have a 450 nm filter. What other filters can I use?** You can use filters with the absorbance between 450 and 490 nm, even though 450 nm filter gives the best sensitivity.