

## CCK-8 Reagent

**Discription:** CCK-8 is a one-bottle solution; no additional reagents are required for assays. 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 an orange colored product (formazan), which is soluble in the tissue culture medium. The amount of the formazan dye generated by dehydrogenases in cells is directly proportional to the number of living cells.

### Content and Storage:

Product Number	Amount
20110	1 mL ( 100 tests)
20118	5 mL ( 500 tests)
20119	10 mL ( 500 tests x 2)
20128	30 mL ( 500 tests x 6)
20158	100 mL (10,000 tests)

*CCK-8 Reagen is supplied in liquid form. Please store the kit at 4°C for frequent use. CCK-8 is stable over one year at 0-5°C with protection from light. Store it at -20°C for longer storage.*

### Short Protocols:

#### **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% CO<sub>2</sub>).
2. 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.*
3. Incubate the plate for 1 - 4 hours in the incubator.
4. Measure the absorbance at 450 nm using a microplate reader.

*To measure the absorbance later, add 10 µl of 1% w/v SDS or 0.1 M HCl to each well, cover the plate and store it with protection from light at room temperature. No absorbance change should be observed for 24 hours.*

#### **Cell Proliferation and Cytotoxicity Assay**

1. Dispense 100 µl of cell suspension (5000 cells/well) in a 96-well plate. Pre-incubate the plate for 24 hours in a humidified incubator (e.g., at 37°C, 5% CO<sub>2</sub>).
2. Add 10 µl 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 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.*
5. Incubate the plate for 1 - 4 hours in the incubator.
6. Measure the absorbance at 450 nm using a microplate reader.

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*To measure the absorbance later, add 10  $\mu$ l of 1% w/v SDS or 0.1 M HCl to each well, cover the plate and store it with protection from light at room temperature. No absorbance change should be observed for 24 hours.*

### **Precautions:**

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. Phenol red containing culture media can be used with this kit for cell viability assays.
5. Membrane filtration is recommended for the sterilization of the CCK-8 solution, if necessary.
6. 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 ( $\sim 10^5$  cells/well) may be necessary.
7. Since the cytotoxicity of this kit is very low, further color development is possible after reading the absorbance.
8. Another reagent for cell proliferation assay such as neutral red or crystal violet can be used after the CCK-8 assay.
9. Measure and subtract the O.D. at 600 nm or higher from that of sample if there is a high turbidity in the cell suspension.

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### **Frequent Asked Questions:**

#### **1. How many cells should there be in a well?**

For adhesive cells, at least 1000 cells are necessary per well (100  $\mu$ l medium). For leukocytes, at least 2500 cells are necessary per well (100  $\mu$ l medium) because of low sensitivity. The recommended maximum number of cells per well for the 96-well plate is 25,000. 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. Since WST-8 and its formazan dye are 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 medium containing phenol red is usable for the CCK-8 assay.

#### **4. Is CCK-8 toxic to cells?**

Since the toxicity of CCK-8 is so low, the same cells can 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.

#### **5. I do not have a 450 nm filter. What other filters can I use?**

You can use filters with the absorbance between 430 and 490 nm, even though 450 nm filter gives the best sensitivity.