

Cell Proliferation and Cytotoxicity Assay with Cell Counting Kit-8

In Brief

Cell Counting Kit-8 (CCK-8) (Catalog # 7368) is a premixed, ready-to-use colorimetric assay used for determining the number of viable cells in a sample. CCK-8 solution utilizes a highly water-soluble WST-8 salt that produces a water-soluble formazan dye upon reduction, in the presence of an electron mediator, 1-methoxy PMS. WST-8 is reduced by dehydrogenases in cells to yield an orange-colored formazan dye, which is soluble in the tissue culture medium. The quantity of formazan dye generated by intracellular dehydrogenase activity is directly proportional to the number of living cells; CCK-8 does not stain viable cells. The excellent stability and low cytotoxicity of the CCK-8 solution also make the kit highly suited for assays that require long incubation (24 to 48 hours). The detection sensitivity of CCK-8 is higher than assays utilizing other tetrazolium salts such as MTT, XTT, MTS or WST-1.

Note:

The recommended maximum number of cells per well for the 96-well plate is 25000. For adhesive cells, at least 1000 cells are necessary per well (100 µL medium). For leukocytes, at least 2500 cells are necessary per well (100 µL medium) because of their low sensitivity. 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.

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.

To sterilize the CCK-8 solution, filter with a 0.2 µm membrane.

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.

If there is a high turbidity in the cell suspension, measure and subtract the O.D. at 600 nm or higher from that of sample.

Storage and Handling

Store at 0 - 5°C. CCK-8 is stable over 1 year at 0 - 5 °C with protection from light. Store it at -20 °C for longer periods. Repeated thawing and freezing causes an increase in the background, which interferes with the assay.

Required Equipment and Materials

- Cell Counting Kit-8
- Plate reader (450 nm filter is optimal, any filter with absorbance between 430 and 490 nm can be used)
- 96-well plate
- CO₂ incubator
- 10 µL and 100-200 µL multi-channel pipettes

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., HeLa Cells at 37°C, 5% CO₂).
2. Add 10 µL of the Cell Counting Kit-8 solution to each well of the plate. Be careful not to introduce bubbles to the wells since they can 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% CO₂).
2. Add 10 µL of various concentrations of substances to be tested to the plate. Incubate the plate for an appropriate length of time (e.g., 6, 12, 24 or 48 hours) in the incubator.
3. 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.
4. Incubate the plate for 1-4 hours.
5. 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.

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NOTICE

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