Cell Counting Kit-8 (CCK-8)

Item NO.	**Product Name**
KTC011001	Cell Counting Kit-8 (CCK-8)

ATTENTION

For laboratory research use only

Not for clinical or diagnostic use

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Background

Cell Counting Kit-8 (CCK-8) allows very convenient assays by utilizing highly water-soluble tetrazolium salt-SST-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 mediator. CCK-8, being nonradioactive, 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. The product (formazan) produced by WST-8 is water soluble, No organic solvents or isotopes required. And the formazan is stable and safe.The detection sensitivity using CCK-8 is higher than assays using other tetrazolium salts such as MTT,XTT, MTS or WST-1.

About This Kit

Cell Counting Kit-8 (CCK-8) is designed to detect cell proliferation and cell toxicity based on WST-8 is reduced by dehydrogenases in cells to give an orange colored product (formazan). The amount of the formazan in cells is directly proportional to the number of living cells.

Kit Component

One-bottle, ready-to-use CCK-8 solution. No premixing of components required.

Cell Number Determination Protocol

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_2).
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 Protocol

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 1-10 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.
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.

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. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.
3. 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.
4. If the color or PH of culture media is changed due to long-time culture, please change the culture media when adding CCK-8.
5. The same cells can be used for other cell assays because of the low toxicity of CCK-8.