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Cell Counting Kit-8

1 Kit Contents

Cat. No.	Product Name	Package
HY-K0301-100T	Cell Counting Kit-8	1 mL
HY-K0301-500T	Cell Counting Kit-8	5 mL
HY-K0301-3000T	Cell Counting Kit-8	5 mL × 6
HY-K0301-12000T	Cell Counting Kit-8	(5 mL × 6) × 4

2 General Information

Cell Counting Kit-8 (CCK-8) provides a tool for studying induction and inhibition of cell proliferation in any *in vitro* model. Cell Counting Kit-8 (CCK-8) allows very convenient assays by utilizing highly 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 mediator, as shown in Figure 1.

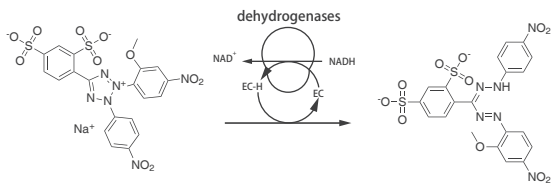

Figure 1 Structures of WST-8 and WST-8 formazan

CCK-8 is a one-bottle solution, ready to use. 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 (Figure 2).

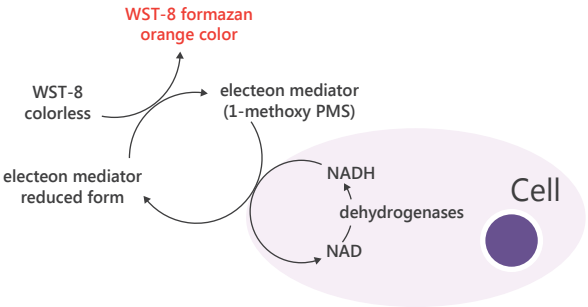

Figure 2 CCK-8 detection mechanism

3 General Protocol

1. Cell Number Determination

a. 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).

b. 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.

c. Incubate the plate for 1-4 hours in the incubator.

d. 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.

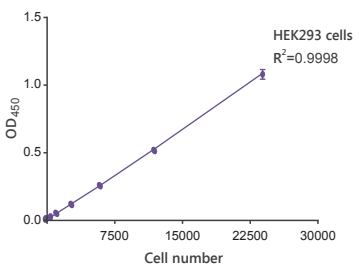

Figure 3 Cell number determination using CCK-8

Cell line : HEK293

Medium : DMEM, 10% FBS

Incubation : 37°C, 5% CO_2, 2 hours

2. Cell Proliferation and Cytotoxicity Assay

a. Seed cells in a 96-well plate at a density of 10^4-10^5 cells/well in 100 μL of culture medium with or without compounds to be tested. Culture the cells in a CO_2 incubator at 37°C for 24 hours.

b. Add 10 μL of various concentrations of substances to be tested to the plate.

c. Incubate the plate for an appropriate length of time (e.g., 6, 12, 24 or 48 hours) in the incubator.

d. Add 10 μL of CCK-8 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.

e. Incubate the plate for 1-4 hours in the incubator.

f. Before reading the plate, it is important to mix gently on an orbital shaker.

g. 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.

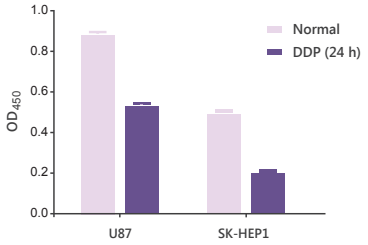

Figure 4 Toxicological test of chemicals using CCK-8

Cell line : U87, SK-HEP1
Medium : DMEM, 10% FBS
Chemicals : 200 μM Cisplatin (DDP)
Incubation : 37°C, 5% CO_2, 2 hours

4 Storage

Store at 4°C 12 months
Store at -20°C 2 years
Protected from light

5 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. If the sterilization of the CCK-8 solution is necessary, please filter the solution with a 0.2 μm membrane.

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

7. This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

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