Product Information

Name: Cell Counting Kit-8

Catalog: C-6005-T (100 assays) 1mL, C-6005 (500 assays) 5× 1mL

Storage and Handling

CCK-8 is stable for 2 years at -20 °C, 1 year at 4 °C and 6 months at room temperature with protection from light. Repeated thawing and freezing causes an increase in the background, which interferes with the assay. To avoid repeated thawing and freezing, keep the kit at 4 °C if it is frequently used.

Description

Cell Counting Kit-8 (CCK-8) utilizes the highly water-soluble tetrazolium salt [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl) -2H-tetrazolium,monosodium salt] to produce a watersoluble formazan dye upon reduction in the presence of an electron carrier.

Cell Counting Kit-8 is a one-bottle solution; no premixing of components is required. Cell Counting Kit-8, being nonradioactive, allows sensitive colorimetric assays for the determination of the number of viable cells in cell proliferation and cytotoxicity assays. CCK 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.

Experimental Protocol

1. Cell Proliferation Assay:

1) Inoculate cell suspension (100 μ l/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% CO2).

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 μ l 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.

2. 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% CO2).

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 450 nm 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. CCK may react with reducing agents to generate CCK 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. 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 (~10^5cells/well) may be necessary.

6. Since the cytotoxicity of this kit is very low, further color development is possible after reading the absorbance. Neutral red or crystal violet can be used after the CCK-8 assay.

7. Measure the reference wavelength at 600 nm or higher if there is a high turbidity in the cell suspension.