Cyrusbioscience

Cell Counting Kit-8 (CCK-8)

Cell Proliferation and Cytotoxicity Assay

GENERAL INFORMATION

Cell Counting Kit-8 (CCK-8) allows very convenient assays by utilizing Dojindo's 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. 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. WST-8 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.

Kit Contents

Size	Reactions
1mL	100 assays
5mL	500 assays
10mL	1000 assays

Storage: 1 year at 4^{o}C with protection from light.

Required Equipment and Materials

plate reader (450 nm filter)

96-well plate

10 µl, 100-200 µl and multi-channel pipettes

CO_2 incubator

Protocol

1. Dispense 100 µl of cell suspension (5000-10000 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_2).
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., 24 or 48 hours) in the incubator.
4. Add 10 µl of CCK-8 solution to each well of the plate.
5. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. Reading.
6. Incubate the plate for 1 - 4 hours in the incubator.
7. Measure the absorbance at 450 nm using a microplate reader.

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 (~105 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. Neutral red or crystal violet can be used after the CCK-8 assay.
9. Measure the reference wavelength at 600 nm or higher if there is a high turbidity in the cell suspension.