CCK-8 Cell proliferation and Cytotoxicity Assay Kit

Cat No: CCK-M-002-1000
Size: 10ml (1000 reactions)
Storage at 2-8°C , protect from light

MOLEQULE-ON®

Description

Cell Counting Kit-8 (CCK-8) allows very convenient assays by 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. CCK-8 is a one-bottle solution; no premixing of components is required. 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.

Protocol

1. Collect cells. Add 100µl Cell suspension（5000-10000 cells) into 96-well plate.
2. Incubate the plate for 24 hrs (at 37°C , 5% CO_2).
3. Add 10 µl of various concentrations of substances to be tested to the plate. Incubate at 37°C .
4. Add 10 µl CCK-8 solution each well. Incubate for 1-4 hour at 37°C .
5. Measure the absorbance at 450nm.

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 (~10 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. Another reagent for cell proliferation assay such as neutral red or crystal violet can be used after the CCK-8 assay.
9. 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.