CellCount™ Cell Counting Kit-8 (CCK-8)

CC02-01/CC02-06/CC02-20

V2.0

Store at 2-8 °C
For Research Use Only

Introduction

CellCount™ Cell Counting Kit-8 (CCK-8) includes a water-soluble tetrazolium compound with high sensitivity and nonradioactive for measuring cell viability. When it is added to the test cells directly, it forms a water-soluble orange formazan dye via an electron carrier. The amount of the formazan dye generated in cells is directly proportional to the number of living cells.
CellCount™ Cell Counting Kit-8 (CCK-8) is a ready to use and fast method to study the cell proliferation and cytotoxicity in medicine treatment. It has excellent stability and little cytotoxicity for living cells when long time procedure is executed. The detection sensitivity of **CellCount™ Cell Counting Kit-8 (CCK-8)** is significantly higher than other assays using the tetrazolium salts such as MTT, XTT, MTS or WST-1.

Product Components

CellCount™ Cell Counting Kit-8 (CCK-8) (CC02-01)		**500 tests**
Cell Counting Kit-8 (CCK-8)	5 mL	1 bottle
User's manual		
CellCount™ Cell Counting Kit-8 (CCK-8) (CC02-06)		**3,000 tests**
Cell Counting Kit-8 (CCK-8)	5 mL	6 bottles
User's manual		
CellCount™ Cell Counting Kit-8 (CCK-8) (CC02-20)		**10,000 tests**
Cell Counting Kit-8 (CCK-8)	5 mL	20 bottles
User's manual		

Safety Information

Please wear gloves, lab coat and goggles while operating. Prevent contact product directly. In case of contacting, wash with large amount of water.

Storage

CellCount™ Cell Counting Kit-8 (CCK-8) should be stored at 2-8 °C and shielded from light. Please use up the product in 12 months. Store at -20 °C for longer storage.

Materials needed but not provided

1. 96 well plate with clear bottom
2. CO_2 incubator
3. Plate Reader capable of measuring absorbance in the region of 450 nm
4. 1% w/v SDS or 0.1M HCl

Instruction

NOTE: If necessary, membrane filtration is recommended for the sterilization of the CCK-8 solution.

A. Cell Number Determination

1. Add 100 µL cell suspension containing known numbers of viable cells into a 96 well plate for a calibration curve.
2. Add 100 µL of the cell suspension to other wells then culture the cells in a CO_2 incubator at 37°C for 24 hours.
3. Thaw the CCK-8 solution on the benchtop or in a water bath at 37 °C if it is frozen.

NOTE: Repeated thawing and freezing of the CCK-8 solution will interfere with the background signal of the assay.

4. Add 10 µL of the CCK-8 solution to each well of the plate and avoid the bubbles to interfere with the optical density reading.
5. Incubate the plate in a CO_2 incubator at 37°C for 1-4 hours.

NOTE: The incubation time can be less than 1 hour or more than 4 hours, depending on the cell type and the cell numbers of well. Optimize the incubation time for each experiment.

8. Measure the absorbance at or near 450 nm on a plate reader. The optical density will be stable for 2 days by adding 10 µL of 1% w/v SDS or 0.1M HCl to each well.

B. Cell Proliferation Assay and Cytotoxicity Assay

1. Prepare a cell suspension using an appropriate culture medium around 10,000-500,000 cells/ml according to cell type.
2. Add 100 µL of the cell suspension to each well of a 96-well plate then culture the cells in a CO_2 incubator at 37°C for 24 hours.
3. Add 10 µL of various concentrations of substances into the test well.
4. Incubate the plate for an appropriate length of time (e.g. 6, 12, 24 or 48 hours) in the CO_2 incubator.
5. Thaw the CCK-8 solution on the benchtop or in a water bath at 37 °C if it is frozen.

> **NOTE:** Repeated thawing and freezing of the CCK-8 solution will interfere with the background signal of the assay.

6. Add 10 µL of the CCK-8 solution to each well of the plate and avoid the bubbles to interfere with the optical density reading.
7. Incubate the plate in the CO_2 incubator at 37°C for 1-4 hours.

> **NOTE:** The incubation time can be less than 1 hour or more than 4 hours, depending on the cell type and the cell numbers of well. Optimize the incubation time for each experiment.

8. Measure the absorbance at or near 450 nm on a plate reader. The optical density will be stable for 2 days by adding 10 µL of 1% w/v SDS or 0.1M HCl to each well.

Troubleshooting

Problem	Possible cause	Remedy
The optical density reading is higher than expected	High background	Avoid repeated thawing and freezing the CCK-8 solution
	Too many cells in a well	Decrease the cell numbers per well or decrease the incubation time of CCK-8 solution
The optical density reading is lower than expected or remains the same as the blank well	Very few cells in a well	Increase the cell numbers per well or increase the incubation time of CCK-8 solution
	CCK-8 solution is not added to the well	Add the CCK-8 solution to each well
	The substances cause cell death	Decrease the concentration of substances

Related Visual Protein Products

CellCount™ MTT Assay Kit	CC01-11	1,000 tests
CellCount™ MTT Assay Kit (with MTT Solvent)	CC01-12	1,000 tests
CellCount™ MTT Assay Kit	CC01-51	5,000 tests
CellCount™ MTT Assay Kit (with MTT Solvent)	CC01-52	5,000 tests
CytoMore™ Cell Rescue Supplement	CT01-1BT	1 bottle
HybriMore™ Hybridoma Culture Supplement	HB01-1L	1 bottle
ImmunoFast™ Adjuvant	IF01-4N	4 reactions
ImmunoFast™ Adjuvant	IF01-20N	20 reactions
Trypan Blue Solution (0.4%)	TPB01-100	100 mL