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

CC02-01/CC02-06/CC02-20

V3.2

Store at -20 °C
For Research Use Only

Introduction

The **CellCount™ Cell Counting Kit-8 (CCK-8)** is a non-radioactive assay kit that uses a water-soluble tetrazolium compound to measure cell viability. When the compound is added directly to cells, an electron carrier converts it into a water-soluble orange formazan dye, the amount of which is directly proportional to the number of living cells. The **CellCount™ Cell Counting Kit-8 (CCK-8)** offers a fast and convenient, ready-to-use method for cell proliferation and cytotoxicity studies. It has excellent stability and little cytotoxicity for living cells during long-term observation. The detection sensitivity of **CellCount™ Cell Counting Kit-8 (CCK-8)** is significantly higher than other similar assays using 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)	CC02-01	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)	CC02-01	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)	CC02-01	5 mL	20 bottles
User's manual			

Safety Information

Please use personal protective equipment such as gloves, lab coat, and goggles when handling. Avoid direct contact with the product content. In the event of contact, wash the affected area with a large amount of water.

Storage

Store **CellCount™ Cell Counting Kit-8 (CCK-8)** at -20 °C and shielded from light. Expiration date is labeled on the bottle or box. Aliquot upon using and store working solutions at 2-8 °C for up to 3 months. Avoid repeated freeze-thaw cycle.

Materials needed but not provided

1. 96 well plate with clear bottom
2. Humidified incubator (e.g. CO_2 incubator)
3. Plate Reader capable of measuring absorbance in the region of 450 nm
4. 1% w/v SDS (Stop solution)

Instruction

NOTE: Sterilize CCK-8 solution by membrane filtration if necessary.

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 humidified incubator at 37 °C for 24 hours.
3. Thaw the CCK-8 solution on the benchtop before use.

NOTE: Thawed CCK-8 solution can be stored at 2-8 °C for 3 months.

NOTE: Repeated freeze-thaw will result in increased background signal affecting assay sensitivity.

4. Add 10 µL of the CCK-8 solution to each well. Avoid introducing bubbles to minimize interference to optical density reading.
5. Incubate the plate in humidified incubator at 37 °C for 1-4 hours.

NOTE: The incubation time can range from less than 1 to more than 4 hours, depending on cell type and cell numbers. Optimize the incubation time for each experiment.

6. 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 (Stop solution) to each well.

B. Cell Proliferation Assay and Cytotoxicity Assay

1. Prepare a cell suspension (10,000-500,000 cells/ml, depending on application and cell type) using an appropriate culture medium.
2. Add 100 µL of the cell suspension to each well of a 96-well plate then culture the cells in humidified incubator at 37 °C for 24 hours.
3. Add test articles at a volume of ~10 µL into the test well.
4. Incubate the plate for an appropriate length of time (e.g. 6, 12, 24 or 48 hours) in the humidified incubator.
5. Thaw the CCK-8 solution on the benchtop before use.

> **NOTE:** Thawed CCK-8 solution can be stored at 2-8 °C for 3 months.
>
> **NOTE:** Repeated freeze-thaw will result in increased background signal affecting assay sensitivity.

6. Add 10 µL of the CCK-8 solution to each well. Avoid introducing bubbles to minimize interference to optical density reading.
7. Incubate the plate in humidified incubator at 37 °C for 1-4 hours.

> **NOTE:** The incubation time can range from less than 1 to more than 4 hours, depending on cell type and cell numbers. 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 (Stop solution) 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
CellCount™ Cell Counting Kit-8 (CCK-8 powder)	CC03-01	500 tests
CellCount™ Cell Counting Kit-8 (CCK-8 powder)	CC03-10	5,000 tests
CytoMore™ Cell Rescue Supplement	CT01-1BT	1 bottle
NeuronMore™ Neural Cell Culture Supplement	CT02-1L	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