FOR RESEARCH USE ONLY

NOT FOR IN VITRO CLINICAL DIAGNOSIS

Cell Counting Kit-8 CCK-8 Assay Kit

Catalog No: FY-C6514 Size: 1000T/10000T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact us.

Phone: 86-027-87002654 86-180 6275 0658

Email: info@feiyuebio.com

Website: www.feiyuebio.com

Intended Use

The kit is intended for use in the determination of the number of viable cells in cell proliferation and cytotoxicity assays.

Principle of the Procedure

Cell Counting Kit-8 (CCK-8) allows very convenient assays by utilizing 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, 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. Cell Counting Kit-8 (CCK-8) is designed to detect cell proliferation and cell toxicity based on WST-8 is reduced by dehydrogenases in cells to give an orange colored product (formazan). The amount of the formazan in cells is directly proportional to the number of living cells. The product (formazan) produced by WST-8 is water soluble, no organic solvents or isotopes required. And the formazan is stable and safe. The detection sensitivity using CCK-8 is higher than assays using other tetrazolium salts such as MTT, XTT, MTS or WST-1.

Limitations of the Procedure

1. This kit is for laboratory scientific research only, we will not be responsible for any consequences if this kit is used for clinical diagnosis or any other procedures.
2. This kit should be used before its expiration date, and please strictly follow the instructions for storage.
3. Different manufacturers' kits or testing the same analyte by other methods may produce inconsistent results because we do not compare our products with those of other manufacturers.
4. In order to get the best experimental results, please use only the reagents provided by the manufacturer, and do not mix reagents from different batches.
5. In order to obtain reproducible results, each step in the experiment should be controlled and variations in sample

collection, handling and storage may also lead to differences in sample measurements.
6. Although each kit passes rigorous quality testing, differences in measured values between batches of kits can still be caused by factors such as shipping conditions and different laboratory equipment.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	1000 T	10000 T	
Ready-to-use CCK-8 solution	10 mL	100 mL	Storage at 4℃ or -20℃

Storage & Stability

1. After receiving the kit, an unopened kit can be stored at 4℃ for 12 months, protected from light. Storage at -20℃ for a more long time, avoid repeated freeze-thaw cycles. If you use it frequently, store the kit at 4℃.
2. Reagent bottle cap must be tightened to prevent evaporation and microbial pollution.
3. The expiration date of the product is determined by the label on the box.

Materials & Equipment Required But Not Provided

Microplate Reader capable of measuring absorbance at OD450 nm
96 well plate with clear flat bottom, Precision pipettes, disposable pipette tips
Humidified incubator (e.g., at 37℃, 5% CO_2).

Note for Procedure

1、Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories.
2、Do not mix or use components from other lots. The kit should not be used beyond the expiration date on the kit label.
3、To avoid cross-contamination, change pipette tips between additions of each sample additions.

Reagent Preparation

Ready-to-use CCK-8 solution: Ready-to-use, no premixing of components required.

Assay Protocol

Cell Number Determination Protocol
1. Inoculate cell suspension (100 µL/well) in a 96-well plate. Pre-incubate the plate in a humidified incubator (e.g., at 37℃, 5% CO_2).
2. Add 10 µL 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 OD reading.
3. Incubate the plate for 1-4 h in the incubator. The incubation time depends on the experimental conditions such as cell type and cell density.
4. Measure the absorbance at 450 nm using a microplate reader.

Cell Proliferation and Cytotoxicity Assay Protocol
1. Dispense 100 µL of cell suspension (5000 cells/well) in a 96-well plate. Pre-incubate the plate for 24h in a humidified incubator (e.g., at 37℃, 5% CO_2).
2. Add 1-10 µL of various concentrations of substances to be tested to the plate.
3. Incubate the plate for an appropriate length of time (e.g.,6, 12, 24 or 48 h) in the incubator.
4. 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 OD reading.
5. Incubate the plate for 1-4 h in the incubator. The incubation time depends on the experimental conditions such as cell type and cell density.
6. Measure the absorbance at 450 nm using a microplate reader

Note for Assay Protocol:

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、Be careful not to introduce bubbles to the wells, since they interfere with the OD reading.

3、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 h) or a large number of cells (~105 cells/well) may be necessary.

4、If the color or pH of culture media is changed due to long-time culture, please change the culture media when adding CCK-8.

5、The same cells can be used for other cell assays because of the low toxicity of CCK-8.

Typical Data

Typical standard curve:

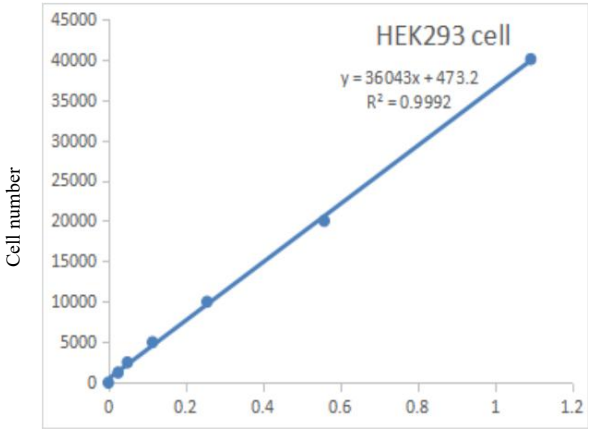

Fig. The CCK-8 kit for 96-well plate analysis detects cell viability. Data provided for demonstration purposes only. A new standard Curve must be generated for each assay.