Ultra Cell Counting Kit-8(CCK-8)Assay Kit

(RCK001 Size:500T/1000T/10000T)

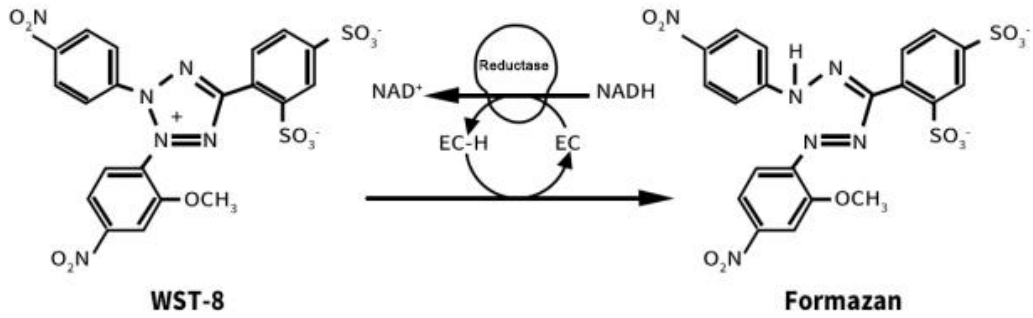
Intended Use

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

The ultrasensitive CCK-8 kit is a rapid, highly sensitive colorimetric assay widely used for cell proliferation and cytotoxicity detection. It is based on WST-8 (chemical name: 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt).

Detection Principle:

In the presence of the electron mediator 1-Methoxy PMS, WST-8 can be reduced by certain mitochondrial dehydrogenases to produce a water-soluble orange-yellow formazan (see Figure 1). The intensity of the formazan color is directly proportional to cell proliferation and inversely proportional to cytotoxicity. The optical density (OD) is measured at 450 nm using a microplate reader, which indirectly reflects the number of living cells.
Compared to the basic CCK-8 kit, this ultrasensitive version offers higher sensitivity, significantly stronger signal, and rapid reaction (most cells show noticeable color change after approximately 30 minutes of incubation). It also has a broader linear range, effectively improving experimental efficiency. It is suitable for assays of cell proliferation, cytotoxicity, drug screening, and inhibition of cell growth.



(Figure 1: WST-8 Molecular Structure and Detection Principle)

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
	500 T	
Ready-to-use CCK-8 solution	1 mL*5	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, the volume of imeasuring equipment instead of directly pouring into the vials.
3. The expiration date of the product is determined by the label on the box.

Materials & Equipment Required But Not Provided

96-well microplate

CO_2 incubator

Pipettes: 10 µL, 100–200 µL, and multichannel

Microplate reader (450 nm filter)

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.

Procedure:

1. Standard Curve Preparation:

a. Count cells in the prepared suspension using a hemocytometer.

b. Dilute the cell suspension in a series of concentrations with culture medium, typically creating 5–7 gradients with 3–6 replicates per group. Seed cells accordingly.

c. After 2–4 hours of incubation to allow cells to adhere, add 10 µL of CCK-8 reagent to each 100 µL of medium. Incubate for 0.5–2 hours, then measure absorbance at 450 nm.

d. Plot a standard curve with cell number on the x-axis and OD value on the y-axis. This curve can be used to estimate cell numbers in unknown samples, provided the experimental conditions are consistent.

2. Cell Viability Assay:

a. Seed 100 µL of cell suspension per well in a 96-well plate and pre-incubate for 24 hours (37°C, 5% CO_2).

b. Add 10 µL of CCK-8 solution to each well (avoid bubbles and mix gently).

c. Incubate for 0.5–2 hours at 37°C.

d. Measure absorbance at 450 nm.

3. Cell Proliferation / Cytotoxicity Assay:

a. Seed 100 µL of cell suspension per well in a 96-well plate and pre-incubate for 24 hours (37°C, 5% CO_2).

b. Add 10 µL of test substance at the desired concentrations to each well.

c. Incubate for an appropriate time depending on the test substance (e.g., 6, 12, 24, or 48 hours).

d. Add 10 µL of CCK-8 solution to each well, mix gently.

e. Incubate for 0.5–2 hours at 37°C.

f. Measure absorbance at 450 nm.

Calculations:

Cell viability (%) = [(OD_experimental − OD_blank) / (OD_control − OD_blank)] × 100%

Inhibition rate (%) = [(OD_control − OD_experimental) / (OD_control − OD_blank)] × 100%

Definitions:
Experimental well: Contains cells, medium, CCK-8, and test substance.
Control well: Contains cells, medium, and CCK-8 only.
Blank well: Contains medium and CCK-8 only.

Notes and Precautions:

1.For research use only; not for clinical diagnostics or therapeutic purposes.

2.Optimize cell seeding density and incubation time after adding CCK-8. For standard 96-well plates, adherent cells should be ≥1,000 cells/well (100 µL medium), and suspension cells ≥2,500 cells/well (100 µL medium). It is recommended to test several cell density gradients prior to the main experiment. After adding CCK-8 (10% of culture volume), incubate at 37°C and measure OD at 450 nm. Some cells can be detected as early as 0.5 hours.

3.The assay depends on dehydrogenase activity. High concentrations of reducing agents or antioxidants in the system can increase background OD, affecting accuracy. If present, replace the medium before adding CCK-8 or subtract the blank OD of the medium containing the test substance.

4.Metal ions in drugs (e.g., Pb^{2+}, Fe^{2+}, Cu^{2+}) may interfere with color development and reduce sensitivity.

5.If culture medium color changes due to long incubation, wash cells and replace the medium before adding CCK-8. Phenol red does not affect the assay.

6.To ensure homogeneous mixing and reduce reagent residue on pipette tips, dilute CCK-8 with medium before adding.

7.If OD measurement is not immediate, add 0.1 M HCl or 1% w/v SDS to each well. Store at room temperature in the dark; measure within 24 hours. The OD will remain stable. (Use the same volume as the CCK-8 solution.)

8.If OD is low, increase cell number or extend incubation time with CCK-8.