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

(FY-C6514 Size:500T/1000T/10000T)

The CCK-8 Cell Counting Kit is a rapid, highly sensitive colorimetric assay widely used for the evaluation of cell proliferation and cytotoxicity. The assay is based on WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt), a water-soluble tetrazolium salt.

Principle of the Assay

In the presence of the electron-coupling reagent 1-Methoxy PMS, WST-8 is reduced by intracellular dehydrogenases in metabolically active cells to generate a water-soluble orange-colored formazan dye (Figure 1). The amount of formazan produced is directly proportional to the number of viable cells. Consequently, the color intensity increases with cell proliferation and decreases with cellular cytotoxicity.

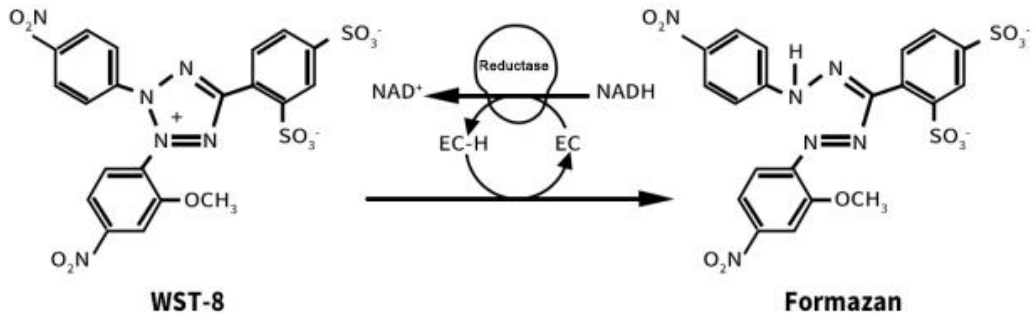
The absorbance (OD value) is measured at 450 nm using a microplate reader, providing an indirect but accurate quantification of viable cell numbers.

Applications

The CCK-8 assay kit is suitable for a wide range of applications, including:

Cell proliferation assays
Cytotoxicity evaluation
Drug screening and efficacy studies
Cell growth inhibition analysis
Assessment of cell viability and metabolic activity

With its high sensitivity, simplicity, and convenience, the CCK-8 assay has become one of the most widely adopted methods for cell-based research and drug discovery.



(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, Store at 4°C for short-term storage. Shelf life: 6 months.Store at −20°C for long-term storage. Shelf life: 24 months.

Note: Repeated freeze–thaw cycles may increase the background OD value and affect assay performance. Avoid multiple freeze–thaw cycles whenever possible.

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)

Assay Procedure

The following protocol is based on a standard 96-well microplate format.

1.Seed 100 µL of cell suspension into each well of a 96-well plate and incubate in a cell culture incubator for 24 hours to allow cell attachment and recovery.

2.Add 10 µL of the test compound at the desired concentration to each well.

Note: If only cell viability is being assessed, Steps 2 and 3 may be omitted. Proceed directly to Step 4.

3.Incubate the plate for an appropriate period according to the experimental design and the characteristics of the test compound.
4.Add 10 µL of CCK-8 solution to each well. Avoid generating bubbles and gently mix the contents to ensure uniform distribution.

5.Incubate the plate in a cell culture incubator for 0.5–2 hours (optimal incubation time may vary depending on cell type and density).
6.Measure the absorbance at 450 nm using a microplate reader.

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.

Cell Viability Calculation

Cell Viability (%) = (As−Ab)/(Ac−Ab) × 100%

Inhibition Rate (%) = 1−(As−Ab)/(Ac−Ab) × 100%

As = Absorbance of the sample wells (containing cells, culture medium, CCK-8 solution, and test compound/drug)
Ac = Absorbance of the control wells (containing cells, culture medium, and CCK-8 solution, but without the test compound/drug)
Ab = Absorbance of the blank wells (containing culture medium and CCK-8 solution, but without cells or test compound/drug)

For clarity in reports or protocols, you may also write:

As: Sample well absorbance
Ac: Control well absorbance
Ab: Blank well absorbance

Precautions and Notes

1.This product is intended for research use only (RUO) and is not intended for use in clinical diagnosis, treatment, or any other medical applications.

2.It is recommended to optimize both the cell seeding density and the incubation time after addition of the CCK-8 solution before conducting formal experiments. For a standard 96-well plate, a minimum of 1,000 adherent cells/well (100 μL culture medium) or 2,500 leukocytes/well (100 μL culture medium) is recommended. Prior to the experiment, it is advisable to prepare a series of wells with different cell densities to determine the optimal assay conditions. Cell culture duration and treatment procedures should be adjusted according to the experimental design. After adding CCK-8 solution (at a volume equivalent to 10% of the culture medium volume per well), incubate the plate at 37°C and measure the absorbance at 450 nm at different time points. Due to the high sensitivity of the CCK-8 assay, some cell types may be measured as early as 0.5 hour after reagent addition.

3.The CCK-8 assay relies on a dehydrogenase-catalyzed reaction. High concentrations of reducing agents (or antioxidants) in the test system may increase the background OD value and interfere with assay results. If reducing agents are present, it is recommended to evaluate the background absorbance and, where possible, eliminate their interference. For example, the culture medium may be replaced with fresh medium before adding the CCK-8 solution to remove the effects of the test compound. If the interference is minimal, medium replacement may not be necessary, and the absorbance of a corresponding blank control containing the test compound can be subtracted during data analysis.
4.In drug inhibition studies, compounds containing metal ions such as Pb^{2+} , Fe^{2+} , Cu^{2+} , and other metals may affect the color development reaction of the CCK-8 solution, resulting in reduced assay sensitivity.

5.If cells have been cultured for an extended period and the culture medium has changed color, it is recommended to wash the cells and replace the medium before performing the CCK-8 assay. The presence of phenol red in the culture medium does not affect the assay results.

6.To ensure thorough mixing with the culture medium and to minimize reagent loss due to adsorption to pipette tips, it is recommended to dilute the CCK-8 reagent with culture medium prior to addition and mix thoroughly before dispensing.

7.If OD measurement cannot be performed immediately, add either 0.1 M HCl solution or 1% (w/v) SDS solution to each well and store the plate at room temperature protected from light. Under these conditions, absorbance values remain stable for up to 24 hours. When using SDS, add a volume equal to the volume of CCK-8 solution added to the well.

8.If the absorbance value is too low, the signal can be improved by increasing the number of cells seeded per well and/or extending the incubation time after addition of the CCK-8 solution.