Enhanced CCK-8 Kit

Catalogue Information

Catalogue No.	Product Name	Pack Size
WS BL1055B	Enhanced CCK-8 Kit	500 tests, 5 × 1 mL
WS BL1055C	Enhanced CCK-8 Kit	1000 tests, 1 × 10 mL
WS BL1055D	Enhanced CCK-8 Kit	2500 tests, 25 × 1 mL
WS BL1055E	Enhanced CCK-8 Kit	10000 tests, 100 mL

Product Description

The Enhanced CCK-8 kit is a rapid and highly sensitive assay based on WST-8, used for detecting cell proliferation and cytotoxicity. Compared to standard CCK-8 kits, it offers higher sensitivity and a wider linear detection range. WST-8 is reduced by cellular dehydrogenases in the presence of 1-Methoxy PMS to form an orange, water-soluble formazan dye. Color intensity correlates linearly with viable cell number.

Usage Instructions

1. Seed the cell suspension into a 96-well plate at: 100 µL per well
   Typical seeding densities:
   a. ~2,000 cells/well for cell proliferation assays
   b. ~5,000 cells/well for cytotoxicity assays
   However, the optimal number of cells per well should be determined based on:
   - Cell size
   - Cell type
   - Proliferation rate (fast vs slow growing cells)
   - Experimental duration

   👉 Important consideration:
   Cells should be seeded within the linear detection range of the assay. It is recommended to perform a preliminary optimization experiment to determine the appropriate seeding density.

2. Treatment / Stimulation
   According to the experimental design:
   - Culture the cells under appropriate conditions
   - Add 0–10 µL of test compound (e.g., drug, stimulant, inhibitor)

   Incubate for a suitable duration, for example:
   - 6 hours
   - 12 hours
   - 24 hours
   - 48 hours

3. Addition of CCK-8 Reagent
   Add CCK-8 solution directly into each well: Standard condition: **10 µL per well (10% of culture volume). Maintain a 1:10 ratio** (CCK-8 : culture volume)

4. Control Setup (Critical for data accuracy)
   Include the following controls:
   - Blank control contains: Culture medium, CCK-8 reagent, Does NOT contain cells. Used to correct background absorbance.
   - Drug interference control contains: Culture medium, CCK-8 reagent, Drug, Does NOT contain cells. Used to correct drug driven variables

5. Incubation with CCK-8
   Incubate the plate in a cell culture incubator for 1-4 hours. The optimal incubation time should be determined experimentally. Pilot experiment may measure absorbance at
   a. 0.5 hours

b. 1 hour
c. 2 hours
d. 4 hours

Select a time point where the signal is strong enough and still within linear range (no saturation)

6. Absorbance Measurement
   Measure absorbance using a microplate read at the primary wavelength of **450nm.** Alternative wavelength include **420nm-480nm**.
   For reference wavelength which will improve the accuracy, particularly for samples with **high turbidity, cell suspensions and background interference**. Use a reference wavelength of **>600nm (e.g. 650nm)**
   Dual wavelength measurement:
   a. OD450 (signal)
   b. OD650 (background correction)

Data analysis

1. Cell Viability Calculation

$$\text{Cell Viability } (\%) = \frac{OD_{\text{treated}} - OD_{\text{blank}}}{OD_{\text{control}} - OD_{\text{blank}}} \times 100\%$$

2. Inhibition Rate Calculation

$$\text{Inhibition Rate } (\%) = \frac{OD_{\text{control}} - OD_{\text{treated}}}{OD_{\text{control}} - OD_{\text{blank}}} \times 100\%$$

3. Definitions

OD (treated)
Absorbance value of wells containing:
- Cells
- Culture medium
- CCK-8 reagent
- Test compound (e.g., drug)

Represents cell response under treatment condition

OD (blank)
Absorbance value of wells containing:
- Culture medium
- CCK-8 reagent
- No cells

Represents background signal

OD (control) (OD(0 treatment))
Absorbance value of wells containing:
- Cells
- Culture medium
- CCK-8 reagent
- No test compound

Represents maximum cell viability (100% reference condition)

Note:
1. Evaporation control in 96-well plates. When using a 96-well plate, especially for long incubation periods, take note of evaporation effects.
2. Optimization of incubation time. The optimal incubation time depends on cell types and number of cells seeded per well. It is highly recommended to perform a preliminary experiment to determine the optimal cell seeding density and optimal incubation time after adding CCK-8 reagent.

3. Ensure that the number of cells in each well is consistent Mix the cell suspension frequently during platting. This will prevent the cell settling which may lead to uneven distribution. After adding CCK-8, gentle shake the plate in all directions several times to ensure that thorough mixing of medium and reagent.
4. This assay depends on dehydrogenase-mediated reactions. If the test substance has oxidizing or reducing properties, it may interfere with the assay signal. Solution include to replace with fresh culture medium before adding CCK-8. If the interference is minimal, use background subtraction (drug-containing blank wells)
5. Medium condition. Prolong incubation may cause the culture medium to change colour or lead to pH variation. Replace with fresh medium before adding CCK-8
6. Avoid bubbles. Before reading absorbance, ensure there are no bubbles in the wells. The bubbles will significantly interfere with OD measurements.
7. Sterility. This kit is produced under sterile conditions. During use, perform all procedures under aseptic conditions and in a biosafety cabinet.
8. Laboratory safety. For personal safety, please wear appropriate laboratory attire and use disposable gloves during operation.

Storage Conditions

Transport with ice pack. Store at 4 °C, tightly sealed and protected from light.
Stable for one year under recommended storage conditions (-20 °C) .
Important to avoid repeated freeze-thaw cycles, as this may degrade reagent performance

Notice

For in vitro research use only, not for diagnostic or therapeutic use. This product is not a medical device.
Contact:

- Support@darwinscience.com