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Ver.250601

## Cell Counting Kit-8 (CCK-8)

CA1210 (100T)

**FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS**

## Product Description

Cell Counting Kit-8, referred to as CCK-8 kit, is an alternative to the MTT method and is based on WST (water-soluble tetrazolium salt, chemical name:2-(2-Methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfobenzene)-2H-tetrazole monosodium salt) widely used for rapid cell proliferation and cytotoxicityHigh-sensitivity detection kit. Can be used for drug screening, cell proliferation determination, cytotoxicity determination, tumour drug sensitivity and other tests.

## Kit components

Components	Volume	Storage
Cell Counting Kit-8	1mL	-20°C, in dark

**Note:** Make aliquots to avoid repeated freeze thaws cycle

## Operation Procedures

### *Cell Viability Test*

1. Plate the cells in a 96 well plate and incubate till it reaches desired confluency.
2. Add 10 $\mu$ L of CCK-8 solution to each well (be careful not to generate bubbles in the well, they will affect the OD reading).
3. Incubate the culture plate in the incubator for 1-4 hours.
  - The incubation time may vary from 30 minutes to overnight depending on the cell type and cell concentration used. Optimize the incubation time for your experiment.
4. Measure the absorbance at 450nm with a microplate reader.
  - If absorbance cannot be measured immediately, add 10 $\mu$ L of 0.1M HCl or 1% SDS(W/V) solution to each well, and cover the culture plate to avoid light and store at room temperature. The absorbance will not change within 24 hours

### *Cell proliferation- toxicity test*

1. Plate the cells in a 96 well plate and incubate till it reaches desired confluency for introduction of test substance.
2. Add10 $\mu$ L of the test substance of different concentrations to the culture plate. Incubate in an incubator for an appropriate period of time (for example: 6,12, 24,or 48 hours).
3. Add 10 $\mu$ L of CCK-8 solution to each well (be careful not to generate bubbles in the well, they will affect the OD reading)
  - If the substance to be tested is oxidizing or reducing, replace the fresh medium before adding CCK-8 (remove the medium, wash the cells twice with the medium, and then add the new medium) to remove the influence of the drug.
4. Incubate the culture plate in the incubator for 1-4 hours.
5. Measure the absorbance at450nm with a microplate reader.
  - If absorbance cannot be measured immediately, add10 $\mu$ L of 0.1MHCl or 1% SDS (W/V) solution to each well, and cover the culture plate to avoid light and store at room temperature. The absorbance will not change within 24 hour

### ***Calculation***

$$\text{Cell viability (\%)} = [A_{\text{test}} - A_{\text{blank}}] \div [A_0 - A_{\text{blank}}] \times 100$$

$A_{\text{test}}$ : absorbance of wells with cells, test substance and CCK-8 solution

$A_{\text{blank}}$ : absorbance of wells with test substance and CCK-8 solution but no cells

$A_0$ : absorbance of wells with cells and CCK-8 solution but no test substance

### **Note:**

The protocol must be standardized at the user's end. The above mentioned protocol is for providing a general outline.