Technical Manual

Enhanced Cell Counting Kit 8 (WST-8 / CCK8)

- Catalogue Code: MAES0207
- Research Use Only

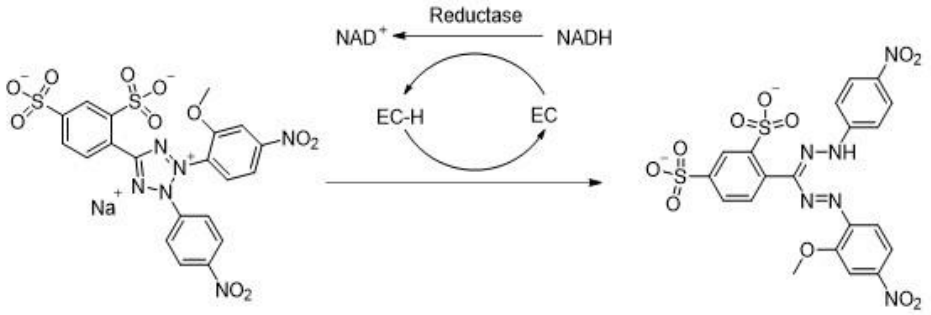
Introduction

The Assay Genie Enhanced Cell Counting Kit 8 (WST-8 / CCK8) is a rapid, highly sensitive, non-radioactive colorimetric test kit based on WST-8 and widely used in the detection of cell proliferation and cytotoxicity. WST-8 is an analog of MTT, an upgraded replacement product of MTT. Compared with MTT, WST-8 has a number of advantages. Firstly, the formazan product produced after the reaction is water-soluble and does not require a specific solvent to dissolve it. Secondly, WST-8 is more stable, has a wider linear range, and has higher sensitivity.

Since WST-8 & WST-8 formazan have no cytotoxicity in the cell culture media, additional experiments may be carried out using the same cells from the previous assay. Just wash the cells and continue.

Detection Method

WST-8 is a compound similar to MTT, which can be reduced to orange formazan by some dehydrogenase in mitochondria in the presence of electron coupling reagent. The amount of formazan produced is directly proportional to the number of living cells. By measuring the absorbance at 450 nm, the amount of living cells can be calculated indirectly.



WST-8 **WST-8 Formazan**

Additional materials required:

1. Microplate reader

Storage

Store at 2~8°C for one year in dark or at -20°C for two years.

Assay Procedure

1. Add 100 µL of cell suspension per well to the 96 well microplate. (Set 2 wells as blank wells, do not seed cells but add 100 µL of culture medium). Incubate the cells at 37°C, 5% CO_2 incubator for 24 h.

Notes:

a) For a cell proliferation test, add 100 µL (about 2,000 cells) cell suspension to each well. For cell cytotoxicity test, add 100 µL (about 5,000 cells) cell suspension to each well. The number of cells used in each well depends on the size of the cell and the rate of cell proliferation, etc.

b) The incubation temperature varies with cell types. Generally, 37°C is recommended for mammalian cells. For other species, please choose the appropriate temperature according to cell culture conditions.

2. Add 10 µL of different concentrations of drugs to each well according to the experimental design. (For the wells with cells added in step 1, set 2 wells as control wells. Do not add drugs but add 10 µL of culture medium).

3. Incubate the cells for an appropriate time according to your experimental design at 37°C, 5% CO_2 incubator.

Notes: According to your individual experimental needs, choose the appropriate incubation conditions and time.

4. Add 10 µL of CCK-8 Buffer and incubate the cells at 37°C, 5%CO_2 incubator for 1~4 h.

Notes: According to your individual experimental needs, choose the appropriate incubation conditions and time.

5. Measure the absorbance with microplate reader at 450 nm.

6. Calculation.

$$\text{Cell Survival Rate (\%)} = \frac{\text{ODsample} - \text{ODblank}}{\text{ODcontrol} - \text{ODblank}} \times 100\ \%$$

$$\text{Inhibition Rate} = \frac{\text{ODcontrol} - \text{ODsample}}{\text{ODcontrol} - \text{ODblank}} \times 100\%$$

Note:
ODsample: the OD value of sample well. (Contains cells, culture medium, CCK-8 and drug solution)
ODcontrol: the OD value of control well. (Contains cells, culture medium, CCK-8, no drugs)
ODblank: the OD value of blank well. (Contains cells, culture medium, no CCK-8 and drugs)

Cautions

1. This kit is for research use only.

2. For your safety and health, please take safety precautions and follow the procedures of laboratory reagent operation. Wear laboratory clothes and disposable gloves during operation and avoid direct contact with the human body or inhalation of the body.

3. For long time storage, please store at -20°C. For ordinary usage, please store at 2~8°C. Avoid freeze/thaw cycles.

4. Pay attention to mixing during cell seeding to avoid an unequal number of cells per well due to cell sedimentation.

5. The incubation time of CCK-8 is generally 1-4 hours. It is recommended to take a preliminary experiment to explore the optimal number of cells and the incubation time of CCK-8.

6. CCK-8 has very low toxicity to cells. Due to dehydrogenase in living cells being continuously produced, CCK-8 can continuously react with the dehydrogenase in living cells. So, the colour of the solution will be darker, and the OD value will continue to increase.

7. The phenol red in the medium will not affect the experimental results. The absorbance of phenol red can be eliminated by subtracting the background absorbance in the blank well during calculation, so it will not affect the detection.

8. When using a 96-well plate for cell culture, pay attention to the resulting error caused by water evaporation. It is recommended to discard the outer circle of wells and add PBS, water, or cell culture medium to prevent water evaporation. In addition, the 96-well plate can also be placed in the incubator near the water source.

9. In order to improve the accuracy of results, make sure that there is no bubble in each well when measuring the OD value with the microplate reader, otherwise, it will interfere with the determination. In addition, it is recommended to use a multi-channel pipette to reduce the difference between parallel wells.

10. The detection of this kit relies on the dehydrogenase catalyzed reaction, so reducing agents (such as some antioxidants) will interfere with the detection. If there are many reducing agents in the system to be detected, try to remove them. Or replace the fresh medium before adding CCK-8 to remove the influence of the drug to be tested.

11. If the added medicine contains metal, it will affect colour development. The final concentration of 1 mM ferrous chloride, ferric chloride, and copper sulphate will inhibit 5%, 15%, and 90% of the colour reaction and reduce the sensitivity. If the final concentration is 10 mM, it will be 100% inhibited.