Technical Manual

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

- Catalogue Code: AKES079
- Research Use Only

Introduction

The Assay Genie Enhanced Cell Counting Kit 8 (WST-8/CCK8) is highly sensitive 1-step kit to quantify the number of live viable cells directly in cell culture supernatants & is an excellent choice for proliferation and cytotoxicity studies.

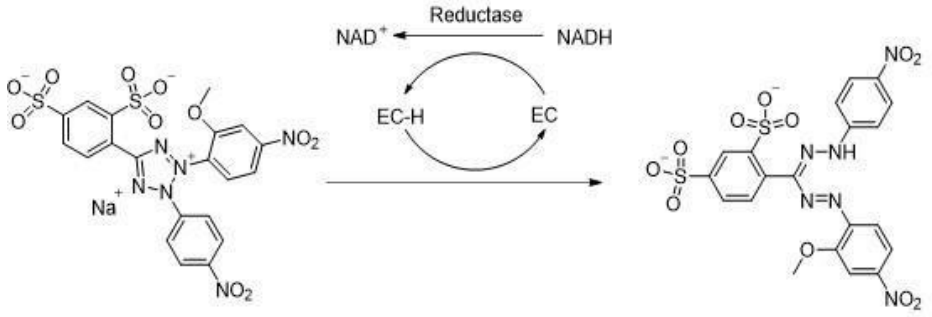
This technology is based on the reduction of WST-8 Tetrazolium salt by mitochondrial dehydrogenases to an orange product which is directly proportional to the number of viable cells when measured at 450nm.

WST-8 is highly soluble in cell culture medium with a low cytotoxicity profile and can be added directly to cultured cells with no pre-treatments or wash steps. It is extremely powerful for sensitive experiments especially those requiring longer incubation steps such as 24 hour or 48 hours.

This kit offers a much higher sensitivity and lower cytotoxicity than other Tetrazolium reagents such as MTS, MTT or XTT

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**

Email: info@assaygenie.com **Web:** www.assaygenie.com

Kit Components

SKU	Product	100 Tests	500 Tests	500 Tests	1000 Tests	10000 Tests
AKES079	Enhanced CCK-8 Buffer	1 mL x 1	5 mL x 1	1 mL x 5	10 mL x 1	50 mL x 2

Additional materials required:

Microplate reader

Storage

Store at 2~8°C for one year away from direct sunlight 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).
   Note: 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.

2. Culture the cells according to the experimental design.

3. Add 10 µL of CCK-8 Buffer and incubate for 1~4 h.
   Notes: CCK-8 incubation conditions are the same as cell culture conditions

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

5. **Calculation**:

$$\text{Cell Survival Rate (\%)} = \frac{OD_{sample} - OD_{blank}}{OD_{control} - OD_{blank}} \times 100\ \%$$

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

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)

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Notes

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 operationand 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 dueto 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 beplaced 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 reducingagents in the system to be detected, try to remove them. Or replace the fresh medium beforeadding 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 10mM, it will be 100% inhibited.