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

Cat. No.: P-CA-001

Size: 100Tests/500Tests/500Tests ×20

Name	100Tests	500Tests	500Tests ×20
Enhanced CCK-8 Buffer	1 mL×1	1 mL×5	1 mL×100

General Information

Product Form Liquid

Sensibility sensitive to light.

Storage Store at 2-8°C or -5~-20°C for two years in dark.

Application 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 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.

Experimental Procedure

1. Add 100 μL of cell suspension per well to the 96 well microplate, and set blank wells (do not seed cells but add 100 μL of culture medium).
   Note: For 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 to each well and incubate for 1-4 h.
   Note: CCK-8 incubation conditions are the same as cell culture conditions.
4. Measure the OD value 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]:

OD_{sample}: the OD value of sample well.

$OD_{control}$: the OD value of control well.

OD_{blank}: the OD value of blank well.

Notes

1. This product is only used for scientific research or further research, not for diagnosis and treatment.
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 -5~-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 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 ofCCK-8.
6. 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.
7. When using a 96-well plate for cell culture, pay attention to the result error caused by water evaporation. It is recommended to discard the outer circle of wells and add PBS, water or culture medium to prevent water evaporation. In addition, the 96-well plate can also be placed in the incubator near the water.
8. 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.
9. 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 reducing agents in the system, try to remove them. Or replace the fresh medium before adding CCK-8 to remove the influence of the reagent.
10. If the added medicine contains metal, it will affect the color reaction. The final concentration of 1 mM ferrous chloride, ferric chloride, and copper sulfate will inhibit 5%, 15%, and 90% of the color reaction. If the final concentration is 10 mM, it will be 100% inhibited.

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