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

Catalog No.:FNCK064
Size: 100T/500T
Applicable samples: Cells
Storage: Store at 2-8℃ away from light for one year and -20℃ away from light for at two years.

Assay Principle:

Enhanced Cell Counting Kit 8 (CCK8), short for WST-8, is a rapid, high-sensitivity test kit based on WST-8 for cell viability, proliferation and cytotoxicity. This kit takes only 0.5 to 1 hour to complete the test, and is faster, more sensitive, and has a wider linear range than conventional or enhanced CCK-8 kits. This kit is suitable for determination of absorbance at around 450nm.

WST-8 is a compound similar to MTT that, in the presence of electron-coupled reagents, can be reduced by some dehydrogenase enzymes within the mitochondria to produce orange-yellow formazan. The more cells proliferate and the faster, the darker the color; The more cytotoxic, the lighter the color. There is a linear relationship between the depth of color and the number of cells.

Compared with WST-1, WST-8 is more sensitive, more soluble, and more stable.

This kit has been optimized to greatly reduce incubation time, generally only 0.5-1 hours to complete the test.

The test kit is very convenient. There is only one tube of enhanced CCK-8 solution that has been prepared, and no further preparation and other operations are required. Without the use of isotopes, all detection steps are performed in the same 96-well plate. It can be used for the detection of large quantities of samples.

Phenol red and serum had no significant effect on the determination of this kit.

This product has no obvious toxicity to cells. After adding enhanced CCK-8 solution for color development, it can be repeatedly read with the enzyme marker at different times, making the detection time more flexible and convenient to find the best determination time.

With a 96-well plate requiring 10μl enhanced CCK-8 solution per 100μl cell, this kit can perform 100 tests per 1ml.

Materials Supplied and Storage Conditions:

Components	100 T	500 T
enhanced CCK-8 solution	1mL	1mL*5

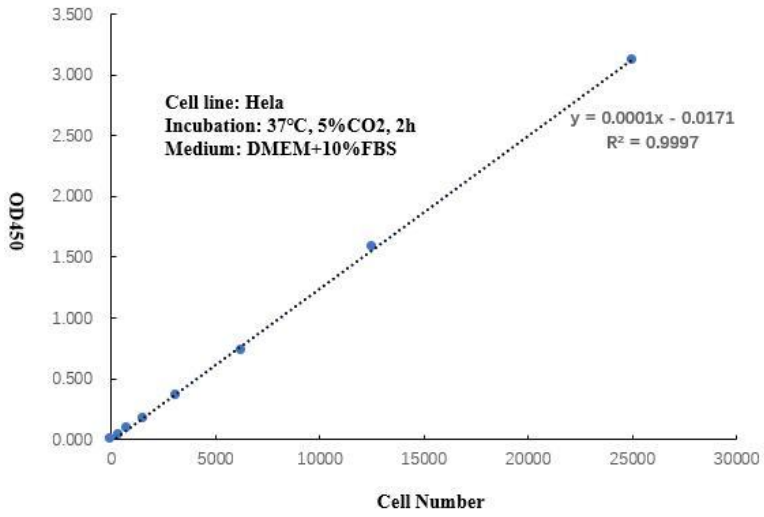
Assay Procedure:

1. Usually 2000 cells with 100 microliters are added to each well for cell proliferation experiments, and 5000 cells with 100 microliters are added to each well for cytotoxicity experiments (the number of cells used in each well depends on the cell culture days, the speed of cell proliferation and other factors). At the same time, a culture medium hole without cells was set up as a negative control, and the cells were cultured according to the cell culture program. If necessary, drug treatment cells can be added.
2. Add 10 microliters of enhanced CCK-8 solution per well. If the initial culture volume is 200 microliters, 20 microliters of enhanced CCK-8 solution should be added, and so on in other cases. If there is concern that the drug used will interfere with the detection, a blank control should be set up in which the appropriate amount of cell culture solution, drug and enhanced CCK-8 solution are added but no cells are added.
3. Continue to incubate in the cell incubator for 0.5-3 hours, for most cases, incubation for 0.5-1 hours is fine.The

length of time depends on the type of cells and the density of cells and other experimental conditions. In the first experiment, the enzyme labeling instrument can be used to detect 0.5, 1, 2 and 3 hours later, respectively, and then a time point with a more appropriate absorbance range is selected for subsequent experiments.

4. Measurement of absorbance at 450nm. If no 450nm filter, 420-480nm filter can be used.

5. Different numbers of HeLa cells were inoculated into 96-well plates according to the culture medium of 100μl per well. After the cells were fully attached to the wall, 10μl enhanced CCK-8 solution was added to each well for incubation for 2 hours, and OD450 was determined.The detection effect is for reference only, and the measured data will be different due to the different detection instruments.



Note:

1. Repeated freezing and thawing of the kit will reduce the detection effect. Although the reagent has no significant effect on its detection effect after repeated freezing and thawing for 3 times, it can be properly packaged and stored after the first thawing in order to achieve good results. In the process of repeated freezing and thawing, there may be a small amount of precipitation, which should be balanced to room temperature and dissolved as far as possible.

2. In most cases, the ideal signal value can be obtained after the addition of enhanced CCK-8 solution to cells by incubation for only 0.5-1 hours, but the incubation time required may be different under different experimental conditions such as cell type and cell density.

3. The signal strength and stability will be affected by the temperature. Before the reaction, the cells and the enhanced CCK-8 solution should be balanced to room temperature before the determination.

4. Due to the use of 96-well plates for detection, if the cell culture time is long, we must pay attention to the problem of evaporation. On the one hand, since a circle around the 96-well plate is the easiest to evaporate, the method of abandoning the circle around the plate can be adopted to add PBS, water or culture solution; On the other hand, the 96-well plate can be placed close to the water source in the incubator to ease evaporation.

5. The detection of this kit depends on the reaction catalyzed by dehydrogenase, so reducing agents (such as some antioxidants) will interfere with the detection. If there are too many reducing agents in the system to be tested, it is necessary to remove them.

6. Ensure that there are no bubbles in each hole before testing with the enzyme marker, otherwise it will interfere with the determination.

7. This product is only used for scientific research by professionals, shall not be used for clinical diagnosis or treatment, shall not be used for food or medicine, and shall not be stored in ordinary homes.

8. For your safety and health, please wear a lab coat and disposable gloves.