Cell-Counting-Kit(CCK-8) Product Instruction

Definition

- Cell counting Kit , an alternative to the MTT method, known as CCK kit for short, is based on WST (water soluble tetrazolium salt, chemical name: 2 – (2 – methyl – 4 – oxygen nitrate phenyl) – 3 – (4 – (phenyl) – 5 – (2, 4 – sulpho benzene) – 2 h –tetrazolium monosodium salt). Nowadays ,it has become a kind of fast high sensitivity detection kit that are widely used in cell proliferation and cytotoxicity tests.
- WST–8 is a compound similar to MTT. When electron coupling reagents exist,it can be reduced to some orange formazan by some dehydrogenase in the mitochondria (see Fig. 1). The more and faster cells proliferate,the darker it gets; The greater the cytotoxicity is, the lighter it gets. For the same cells, the depth of color is linearly related to the number of cells.

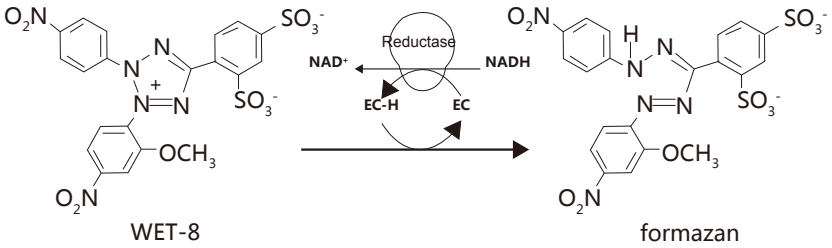

Figure 1. WST-8 detection schematic diagram

Comparison between CCK method and MTT method:

Cell counting method	MTT	CCK-8
Formazan solubility	Bad	Good
Product properties	Powder	Solution
Usage method	Pre configuration	No pre configuration
Detection time	> 4 hours	1-4 hours
Sensitivity	Ordinary	Higher
Detection wavelength	560~600nm	430~490nm
Cytotoxicity	High, The morphology of the cells disappears completely	Low, cell morphology remained unchanged.
Reagent stability	Ordinary	Good
DMSO dissolution needed	Yes	No
Convenience	Ordinary	High

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Protocol

1) Usually in the 96 hole plate, cell proliferation and cytotoxicity test are inoculated with cell suspension (5000 cells and 2000 cells /100 uL/ hole, the specific number of the cells per hole should be decided according to the size and speed of the cell proliferation speed and other factors). The system is filled with a corresponding amount of cell culture medium, drug and CCK–8 solution, and the holes without cells are regarded as a blank control.

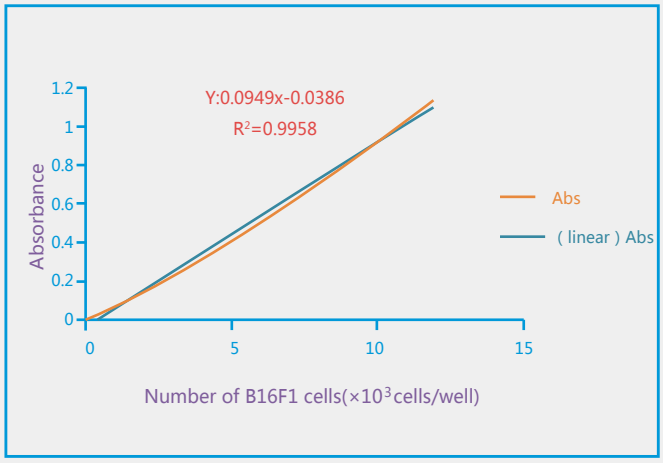
2) The cells adhered to the wall for 24 hours and are given a specific dose of 0–10 mg of stimulation in accordance with the experimental requirements.

3)The drugs are treated for 2~4 days and then add 10 uL of CCK solution at each hole (be careful not to generate bubbles in the pores, which will affect OD value)

4) Incubate the incubator for 1~4 hours, then use the mic–roplate reader to determine the absorbance at 450nm.

5) If you do not detect OD value temporarily, you may add 10 μL 0.1M HCL solution or 1% w/v SDS solution, cover it with culture plate and keep it from light at room tem–perature. Absorbance does not change in 24 hours.

6) The absorbance of phenol red can be eliminated by blank hole so that the result will not be affected.



Vitality calculation

Cell viability * (%) =[A (dosing) –A (blank)]/[A (0 dosing) –A (blank) * 100%

A (dosing): Absorbance of cells, CCK solutions, and drug solutions

A (blank): Absorbance of the culture medium and CCK solution without cells

A (0 dosing): Absorbance of cells, CCK solutions without drug solutions

* Cell viability: Cell proliferation activity or cytotoxicity activity

Storage conditions

4 degrees centigrades for one year; –20 degrees centigrades, protected from light for two years.

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