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## MTT Cell proliferation and cytotoxicity Assay Kit Instruction

Catalog Number:BC024

### 一、 Reagents composition

Reagents	Component	500T	1000T	Storage
Reagent 1	5×MTT	5ml	10ml	4°C and keep avoid light
Reagent 2	Diluent	25ml	50ml	4°C

### 二、 Product Description

MTT tetrazolium is a hydrogen acceptant dye that can be directly added to the cell culture medium and reduced to water insoluble dark purple crystalline product, Formazan, by the succinate dehydrogenase of living cells, but the dead cells can not undergo this reduction. The water-insoluble Purple Crystals produced by MTT can be dissolved in specific solvents, and then the light absorption value can be measured at the wavelength of 570nm (500~600nm) by enzyme-labeled instrument, which can reflect the number and metabolic activity of living cells, it also reflects the cell survival, proliferation, growth and toxicity. For example, the cells that proliferated and grew vigorously reduced more MTT and had higher light absorbency after adding cell growth factors, whereas the cells treated with anti-proliferation and anti-tumor drugs or cytotoxic drugs grew more slowly or became more toxic, the lower the light absorption. It is also used to examine the effects of non-drug stimuli such as physical factors on cell proliferation and viability. Compared with the method of 3H thymine incorporation, the method is simple, rapid and reliable. It does not use radionuclide, and is widely used to detect cell survival, proliferation, growth and toxicity.

### 三、 Self-contained instruments and reagents

Low-speed centrifuge, plate Shaker, enzyme label analyzer (570nm Wavelength) , micropipette, 96-well plate, 1.5 ml centrifuge tube, DMSO

### 四、 Storage

Keep at 4°C, MTT should be kept away from light for half a year

### 五、 Points to attention

- 1、 The suspension cells must be centrifuged before the supernatant can be aspirated with DMSO.
- 2、 The formation of Formazan is not only proportional to the number of living cells, but also affected by the



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time of exposure.

- 3、MTT solution is yellow and should be kept away from light. Long time illumination will lead to failure.

Do not use when the color changes to grayish green. The MTT solution will coagulate at 4°C or lower temperature. It can be used in 20-25°C water bath for a while until it is completely dissolved.

- 4、For your safety and health, please wear lab coat and disposable gloves.

### **六、Operating procedure**

- 1、Cells were added at 100μl/hole in 96-well plate, usually 100μl of 2,000 cells per hole in cell proliferation test, cytotoxicity tests add 100μl of 5000-10000 cells per hole (the number of cells per hole depends on the size of the cell and the rate of cell proliferation) . The cells were incubated at 37°C for 24 hours in a 5% CO<sub>2</sub> cell incubator.
- 2、Add the appropriate concentration of 0~10μl compound.
- 3、Incubate the 96-well plates in a cell incubator at 37°C with 5% CO<sub>2</sub> air and 100% humidity for an appropriate time.
- 4、Dilute 5×MTT to 1× MTT solution with Diluent.
- 5、Add 50μl 1×MTT solution to each hole, and incubated at 37°C for 4 hours and MTT was reduced to Formazan.
- 6、Suck out the supernatant, add 150μl DMSO per hole to dissolve the nail, shake well with a flat shaker.
- 7、At the wavelength of 570nm, the optical density of each hole can be measured by the enzyme-labeled instrument (560-600nm filter can be used if no 570nm filter is available)
- 8、Result Analysis
  - A、**Cell Survival Rate:** The OD value of each test hole was subtracted from the background OD value (complete medium plus MTT, no cells), and the OD value of each repeated hole was averaged ±SD
  - B、The cell survival rate is expressed as T/C%, T was the OD value of the treated cells and C was the OD value of the control cells.

Obtain the drug concentration when T/C =50%(IC<sub>50</sub>) or drug concentration when T/C =10%(IC<sub>90</sub>)