MTT Cell Proliferation and Cytotoxicity Assay Kit

C1375225

Storage 2-8℃. Store in the dark.

Introduction

The MTT cell proliferation and cytotoxicity detection kit can be used for the detection of cell proliferation and cytotoxicity. MTT can be reduced by some dehydrogenases in the mitochondria to form a crystalline, dark purple product called methylene blue. In the presence of a specific solvent, it can be completely dissolved. Then, the absorbance at 570nm wavelength can be measured using an enzyme reader. The more and faster the cell proliferation, the higher the absorbance; the greater the cytotoxicity, the lower the absorbance.

Kit Contents

C1375225	Component	500T	2500T	Storage
C1375225A	MTT	25 mg	125 mg	2-8℃. Store in the dark.
C1375225B	MTT Solvent	5 mL	25 mL	2-8℃.
C1375225C	Formazan Solvent	80 mL	5×80mL	2-8℃. Store in the dark.

Note: 500T is sufficient for 500 assays and 2500T is sufficient for 2500 samples.

Usage method

1. Preparation of MTT solution: Dissolve 25 mg of MTT in 5 mL of MTT solvent to prepare a 5 mg/mL MTT solution. Use it in a sterile environment after filtration through a 0.22 μm filter, or store it at -20°C in the dark. It can also be appropriately aliquoted and stored at -20°C in the dark as needed.
2. Take cells in a good growth state, adjust the cell density, and inoculate 100 μL of cell suspension into each well of a 96-well plate. At the same time, set a blank well (without cells but with the same volume of culture medium).
   Note: Usually, in a cell proliferation experiment, 100 μL of approximately 2000 cells is added to each well, and in a cell toxicity experiment, 100 μL of approximately 5000 cells is added (the exact number of cells used in each well depends on the size of the cells and the speed of cell proliferation, etc.).
3. Cultivate and treat the cells according to the experimental design.
4. Add 10 μL of MTT solution to each well, and incubate in the cell incubator for another 4 hours.
5. For adherent cells, simply remove the old culture medium directly to avoid the pipette tip

from scratching the single-cell layer. For suspended cells, centrifuge to collect the cells and remove the culture medium.

6. Add 150 μL of Formazan Solvent, mix appropriately, and continue incubating in the cell incubator. Until all the formazan is completely dissolved under a normal optical microscope. Usually, incubate at 37°C for about 15-30 minutes, and the purple crystals will all dissolve. If the purple crystals are smaller and fewer, the dissolution time will be shorter. If the purple crystals are larger and more numerous, the dissolution time will be longer. At this time, to accelerate the dissolution, you can shake several times appropriately.
7. Observe under a microscope, and measure the absorbance at 570 nm using an enzyme detector after the formazan is fully dissolved.

Matters needing attention

1. This product is for scientific research use only.
2. For your safety and health, please wear laboratory work clothes and disposable gloves during operation, and follow the laboratory reagent operation procedures.
3. Since the detection is carried out using 96-well plates, if the cell culture time is long, be sure to pay attention to the evaporation problem. On the one hand, since the outer circle of the 96-well plate is the most prone to evaporation, you can adopt the method of discarding the outer circle and adding PBS, water or culture medium; on the other hand, you can place the 96-well plate near the water source inside the incubator to alleviate evaporation.
4. The MTT solvent will solidify at low temperatures. Before use, please let it stand at room temperature or incubate in a water bath at 20-25°C for a moment until it is completely dissolved before using.
5. After preparing the MTT solution, it is yellow and needs to be stored in the dark. Long-term exposure to light will cause it to lose its effectiveness. When the color turns grayish green, do not use it. The MTT solution will solidify at 4°C, in an ice bath, etc., and can be incubated in a water bath at 20-25°C until it is completely dissolved before using.
6. When inoculating cells, pay attention to mixing well to avoid cell sedimentation, which may cause inconsistent cell inoculation amounts in each well.
7. When the Formazan Solvent is frozen or has precipitation, it can be incubated in a 37°C water bath to promote dissolution, and must be used after all is dissolved and mixed.
8. Bacteria, mycoplasma or other microorganisms can damage the MTT tetrazolium ring, and contaminated cells cannot be used for this method of detection.