MTT Cell Viability & Proliferation Assay Kit
Catalog No.: CP001
Size: 500 Tests

Signalway Antibody

FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!
PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

Kit contents:

MTT solution	5ml
Dissolved solution	60ml

Storage:

Keep at 2-8℃, protected from light.

Expiry date:

One year

Introduction :

This kit is widely used in cell proliferation and cytotoxic detection. The mitochondria of the live cells have dehydrogenase-related dehydrogen enzymes, which can restore the yellow MTT to generate crystal-shaped blue-purple Formazan. The dead cells have no enzymes and MTT is not restored. Formazan can be fully dissolved in the existence of specific solvents. Then you can measure the absorbance at the wavelength of 460-630nm. The more the cells are proliferated faster, the higher the absorbance; the greater the cytotoxicity, the lower the absorbance. The sensitivity of this kit is high, the linear range is wide, and it is convenient to use.

Protocol (96-well plate):

Adherent cells:

1. Collect cells on logarithmic phase, add 100ul cell suspension per well, and make the density of the cells to be tested about 2000-10000 per well.

2. 5% CO2, 37℃ incubated, until the cells are paved with the bottom of the well (96-well flat bottom), add the drug with the concentration gradient. In principle, the drug can be added after the adhesion of the cells, or it can be added after two hours, or half a day. Adding drug with generally 5-7 gradients, 100ul per well, and 3-5 duplicated wells.

3. With 5% CO2, 37℃ incubated for 16-48 hours, the cells are observed with inverted microscope.

4. Add 10ul MTT solution per well and continue to culture 4h. If the drug can react with MTT, first centrifuge and then remove the culture medium. Be careful with PBS washing for 2-3 times before adding the culture medium containing MTT.

5. Terminate the culture, and be careful to remove the culture medium in the wells.

6. Add 150ul Dissolved solution per well, and set the low-speed oscillation 10min on the shaker to fully dissolve the crystalline. Measure the light absorption value of each well with the microplate reader at 490nm, 550nm or 570nm.

7. At the same time, set zero wells (culture medium, MTT, Dissolved solution), and control wells (cells, the same concentration of drug dissolving medium, culture medium, MTT, Dissolved solution) with 3-5 duplicated wells.

Suspension cells:

1. Collect cells on logarithmic phase, and adjust cell suspension concentration to 1×10^6 cells/ml. Add non-serum culture medium 40ul, 10ul of drugs to be detected, 50ul of cell suspension (i.e. 5×10^4

cells/well), and a total of 100ul is added to the well on 96-well plate (sterilized water or PBS is filled for marginal wells). Each plate is set with a control well (add 100 ul culture medium).

2. With 5% CO2, 37℃ incubated for 16-48 hours, the cells are observed with inverted microscope.

3. Add 10ul MTT solution per well and continue to culture 4h.

4. Centrifuge (1000 rpm for 10min), and carefully remove the supernate. Add 100ul Dissolved solution per well, and set the low-speed oscillation 10min on the shaker to fully dissolve the crystalline. Measure the light absorption value of each well with the microplate reader at 490nm, 550nm or 570nm.

5、At the same time, set zero wells (culture medium, MTT, Dissolved solution), and control wells (cells, the same concentration of drug dissolving medium, culture medium, MTT, Dissolved solution) with 3-5 duplicated wells.

Analysis of test results:

Cell survival rate：Subtract the OD value of zero well from the OD value of each test well. The OD value of each duplicated wells is mean value ± SD.

The cell survival rate is represented by T/C%, T is the OD value of the cells adding drug, and C is the OD value of the control cells.

Cell survival rate% =（OD value of the cells adding drug / OD value of the control cells）×100