MTT-Cell Based Proliferation/Toxicity Assay Kit

Catalog No.: K017

Size: 500T

Storage: 2-8℃ or -20℃ for 12 months.

Kit components:

Reagents	Quantity	Storage
MTT	25mg	2-8℃ or -20℃, away from light
MTT solvent	5ml	2-8℃ or -20℃
Formazan Dissolving Solution	55ml	2-8℃ or -20℃
Instruction manual	1	

NOT: Dissolve 25mg MTT with 5ml MTT solvent to prepare 5mg/ml MTT solution. After preparation, it can be used, or stored directly at -20 ° C away from light, or stored at -20 ° C away from light after proper packaging as required.; The Formazan Dissolving solution can also be stored at room temperature.

Materials Required but Not Supplied

- ✓ Adjustable pipettes and a repeat pipettor
- ✓ A 96-well plate for culturing cells
- ✓ A 96-well plate reader capable of measuring absorbance at 570 nm
- ✓ Distilled water

Introduction

Measurement of cell viability and proliferation forms the basis for numerous in vitro assays of a cell population's response to external factors. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation.

The key substrate of the kit is:3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide or MTT. MTT is a yellowish solution when dissolved in balanced salt solutions without phenol red and is taken up by cells due to its net positive charge. The tetrazolium ring of MTT (yellow) is reduced to purple formazan crystals by intracellular NAD(P)H-oxidoreductases. The formazan crystals are insoluble in aqueous solution, but become

solubilized in the provided Solubilization Buffer. After solubilization, the resulting purple solution is spectrophotometrically measured.

The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. The number of assay steps has been minimized as much as possible to expedite sample processing. The MTT solution yields low background absorbance values in the absence of cells. For each cell type the linear relationship between cell number and signal produced is established, thus allowing an accurate quantification of changes in the rate of cell proliferation.

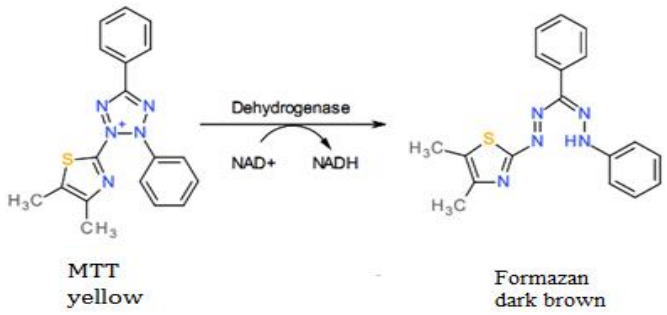

Figure 1: Chemical structure of MTT and its subsequent product. MTT is converted to Formazan.

Procedure: 96 Well Format

If you are familiar with the procedure and know the cell count to use in your specific assay, you may follow this basic protocol.

1、Usually, 2000 cells are added in 100 ul per well for cell proliferation assay, and 5000 cells per well for cytotoxicity asssy (the number of cells used in each well depends on the size of the cells, the speed of cell proliferation, etc.).

2、Incubate for appropriate time according to the experiments and treat with 0-10 ul specific drug.

3、Add 10 μl MTT solution.

4、Incubate the cells for 3-4 hours in the incubator. Dark purple formazan crystals are formed in the cells.

For higher cell density, the incubation time can be shortened accordingly.

5、Take out the Formazan Dissolving Solution in advance and return to room temperature. Add 100 μl Formazan Dissolving Solution and mix appropriately.

6、Incubate for about 4 hours at 37°C until the purple crystals is absolutely dissolved.If the purple crystals are smaller and fewer, the dissolution time will be shorter.If the purple crystal is larger and more, the dissolution time is longer.

7、 Absorbance was measured at 570nm. If no 570nm filter is available, a 560-600nm filter can be used.

Note:

1. Due to the use of 96 - well plates for testing, if the cells are cultured for a long time, the problem of evaporation should be attention. On the one hand, because a circle around the orifice of 96 well is the most easy to evaporate, the circle around can be deprecated or use PBS, water or nutrient solution; On the other hand, the 96 plates can be placed near the water in the cabinet, to alleviate the evaporation.
2. If the Formazan is frozen or produce precipitation, 37℃ water bath can be maintained to promote dissolving, and must be used after dissolving and blending absolutely.
3. MTT solution is yellow, need to avoid light preservation, long light can lead to failure. When the color turned into a sage green, it would not be used.
4. The MTT solvent may be frozen at low temperature, Please place it at room temperature or 20-25℃ until dissolved completely before using.

Performing an assay

The plot of the data obtained in Step 7(absorbance against number of cells) should provide a curve with a linear portion. The optimal number of cells for the assay should fall within the linear portion of the curve and give an absorbance value between 0.75 and 1.25.Then both stimulation and inhibition of cell proliferation can be measured. For best results, cells in log phase of growth should beemployed and the final cell number should not exceed 10^6 cells/cm^2. Each experiment should include a blank containing all of the reagents in a well without cells.

Assays will include:

（1）Blank wells containing medium only.

（2）Untreated control cells.

（3）specific drug treated cells

If more than 100 μl of medium is used per well, increase the amount of MTT solution accordingly, e.g., for 250 μl of medium use 25 μl of MTT solution.

Data interpretation

Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely a higher absorbance rate indicates an increase in cell proliferation.Rarely, an increase in proliferation may be offset by cell death; evidence of cell death may be inferred from morphological changes.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Absorbance readings are too high or too low	A. Cell number is too high or too low B. Cells are contaminated or cells are not healthy	A. Titrate cell density to get an optimal absorbance reading B. Use only healthy cells
Poor consistency of replicates	A. Inaccurate cell seeding B. Inaccurate reagent pipetting	A. Increase cell seeding