MTT-Cell Based Proliferation/Toxicity Assay

Catalog Code: K017

Storage: -20°C dark

MTT is carcinogenic. Avoid direct contact. Use gloves and eye protection. For research use only. Not for human or diagnostic use.

This package insert must be read in its entirety before using this product.

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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.

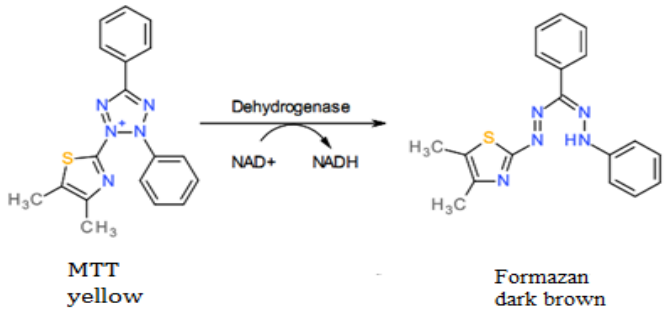

Figure 1: Chemical structure of MTT and its subsequent product. MTT is converted to Formazan.

Materials provided:

Reagents	Quantity	Storage
MTT	25mg	4℃ or -20℃, dark for one year
MTT solvent	5ml	4℃ or -20℃
Formazan Dissolving Solution	55ml	4℃ or -20℃
Instruction manual	1	

NOT: After the preparation of MTT solution, the storage condition was in –20℃ without light;

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
- ✓ For 500 times

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. Seed cells in a 96-well plate at a density of 2000 cells/well (forproliferation Assay) or 5000cells/well (Toxicity Assay)in 100 ul of culture medium (depending on cell type)
2. Incubate for appropriate time according to the experiments and treated with the substance to be assayed.
3. Add 10 µl MTT solution.
4. Incubate the cells for 3-4 hours in the incubator. Dark brown formazan crystals are formed in the cells.
5. For higher cell density, the incubation time can be shortened accordingly.
6. Add 100 µl Formazan Dissolving Solution.
7. Incubate for about4 hours at 37°C when the purple precipitate is absolutely dissolved.
8. Record absorbance at 560-600 nm.

Note:

1. Due to the use of 96 - well plates for testing, if the cellsare 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）Test cells treated with the substance to be assayed.

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