

## MTT Cell Proliferation Assay Kit

### Introduction

MTT Cell Proliferation Assay Kit is a widely used cell proliferation and toxicity assay kit. MTT, a tetrazolium salt, can be reduced by succinate dehydrogenase in the mitochondria of living cells to the water-insoluble purple formazan crystals. The amount of formazan can be quantified by measuring the absorbance at 570 nm upon solubilization and is proportional to the number of living cells.

This kit provides a special formazan solvent, which does not need to remove the original medium and can be directly added to dissolve the formazan. At the same time, this kit is easy to use, highly sensitive and has a good linear range.

### Components and Storage

Components	K2249-500T	K2249-2500T
MTT	25 mg	125 mg
MTT Solvent	5 mL	25 mL
Formazan Solvent	50 mL	250 mL

Store the kit at -20°C, stable for 1 year. MTT should be stored away from light and moisture.

### Protocol

- The MTT working solution preparation:** Dissolve 25 mg MTT in 5 mL MTT Solvent to make a 5 mg/mL MTT working solution. Try to prepare a fresh MTT working solution every time. Or aliquot the MTT working solution into single-use volumes and store them at -20°C away from light, avoiding repeated freeze/thaw cycles, stable for 6 months.

**\*Note:** MTT is light-sensitive and should be kept in the dark. So, it is suggested to cover MTT bottles with foil.

- Cell culture:** For 96-well plates, seed cells at a density of 2000 cells/well for cell proliferation assay or 5000 cells/well for cytotoxicity assay in 100  $\mu$ L culture medium. Treat cells with the interested drug for a desired period. Prepare parallel wells as the background control (only containing medium) and negative control (containing medium, cells and the same volume of solvent for the interested drug).

**\*Note:** The optimal number of cells seeded in each well varies depending on the cell types.

- MTT incubation:** Add 10  $\mu$ L MTT working solution per well, and incubate at 37°C for 4 h.

**\*Note:** The optimal incubation time varies depending on the cell types.

- 4. Formazan dissolution:** Add 100  $\mu$ L Formazan Solvent per well, mix gently (avoid bubbles) and incubate at 37°C for 3-4 h.

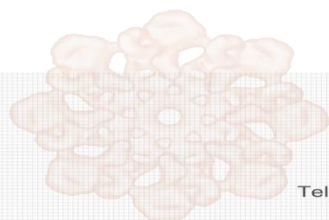
**\*Note:** The optimal dissolution time of formazan varies depending on the cell types. In general, the purple crystals can be observed to be completely dissolved under the microscope after 3-4 h. If the crystallization dissolution is not sufficient, the plate can be shaken on an orbital shaker to accelerate the crystallization dissolution.

- 5. Detection:** Measure the absorbance (A) at 570 nm with a microplate reader. If the 570 nm filter is not available, a 560-600 nm filter can also be used.
- 6. Analysis:** Calculate cell viability with the following equation

$$\text{Cell viability (\%)} = [(A_{\text{Treatment sample}} - A_{\text{Background control}}) / (A_{\text{Negative control}} - A_{\text{Background control}})] \times 100$$

## Note

- When cells are cultured for long periods, the corner and edge wells of the 96-well plate are prone to liquid evaporation. It is recommended to fill the surrounding moat with sterile water, medium, or PBS. Meanwhile, place the plate near the water source in the incubator.
- MTT Solvent may solidify at low temperatures, please equilibrate to room temperature or a 20-25°C water bath for a few moments until completely thawed before using.
- MTT is carcinogenic, so please be careful when using it. At the same time, sterility is required during the MTT assay, because bacteria can also cause the absorbance to rise, which can affect the experimental results.
- The MTT working solution is yellow, which is light-sensitive and should be kept in the dark. If the MTT working solution turns gray-green, it means that it has deteriorated, do not use it at this time. MTT working solution may solidify at low temperatures, please warm to room temperature or 20-25°C water bath for a while until completely thawed before using.
- Formazan Solvent may solidify and occur precipitation. Please warm to room temperature or 37°C water bath for a moment until completely dissolved before using.
- Avoid bubbles or foaming when mixing the Formazan Solvent and medium.
- For your safety and health, please wear lab coats and gloves during the experiment.
- For research use only. Not to be used in clinical diagnostic or clinical trials.



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