# MTT Cell Proliferation and Cytotoxicity Assay Kit

**Cat. No: E-CK-A341** **Size: 500 Assays/1000 Assays**

| Cat. | Products | 500 Assays | 1000 Assays | Storage |
|---|---|---|---|---|
| E-CK-A341A | MTT (5×) | 5 mL×1 | 10 mL×1 | 2~8℃, shading light |
| E-CK-A341B | MTT Diluent Buffer | 12.5 mL×2 | 12.5 mL×4 | 2~8℃ |
| E-CK-A341C | Formazan Dissolution Buffer | 50 mL | 50 mL×2 | 2~8℃, shading light |
| | Manual | | One Copy | |

## Storage

Store at 2~8℃ for one year, MTT (5×) should be stored in dark.

## Introduction

Elabscience® MTT Cell Proliferation and Cytotoxicity Assay Kit is a rapid and highly sensitive kit widely used in cell proliferation and cytotoxicity detection.

MTT is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide which can be reduced by some dehydrogenases in mitochondria to form a crystalline dark purple product formazan. Formazan can be completely dissolved by Formazan Dissolution Buffer and its absorbance is near 570 nm wavelength. The more and faster the cells proliferate, the darker the color is, and the more cytotoxic, the lighter the color is. For the same type of cell, there is a linear relationship between the absorbance and the number of live cells.

## Staining Procedure

**Method 1: For suspension cells and adherent cells**

1. Reagent Preparation
   MTT (5×) is concentrated, please diluted with MTT Diluent Buffer to 1×MTT working solution before use.
   For example: take 100 µL MTT (5×), add to 400 µL MTT Diluent Buffer, and the mixture is 1× MTT working solution.
   Note: Prepare the fresh solution before use. 1×MTT working solution should be stored in dark.
2. Add 100 µL of cell suspension to each well of the 96 well microplate, and set blank wells (no cells but add 100 µL of culture medium).
   Note: For cell proliferation test, add 100 µL (about 2,000 cells) cell suspension to each well. For cell cytotoxicity test, add 100 µL (about 5,000 cells) cell suspension to each well. The seeding cells in each well depends on the size of the cell and the rate of cell proliferation, etc.
3. Culture the cells according to the experimental design.
4. Add 50 µL of 1× MTT working solution to each well and incubate for 4 h.
   Note: MTT incubation conditions are the same as cell culture conditions.


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5. Bring the Formazan Dissolution Buffer to room temperature in advance. Add 100 μL Formazan Dissolution Buffer to each well, then incubate for 1~4 h, so that the formazan can be fully dissolved (to promote the dissolution of formazan, it can be incubated in an incubation shaker at 37 ℃).

6. Observed under the microscope, measure the OD value with microplate reader at 570 nm after the formazan was fully dissolved.

7. Calculation.

$$\text{Cell Survival Rate (\%)} = \frac{OD_{sample} - OD_{blank}}{OD_{control} - OD_{blank}} \times 100\%$$

$$\text{Inhibition Rate} = \frac{OD_{control} - OD_{sample}}{OD_{control} - OD_{blank}} \times 100\%$$

**[Note]:**

$OD_{sample}$: the OD value of sample well.

$OD_{control}$: the OD value of control well.

$OD_{blank}$: the OD value of blank well.

**Method 2: For adherent cells**

1. Reagent Preparation
   MTT (5×) is concentrated, please diluted with MTT Diluent Buffer to 1× MTT working solution before use.
   For example: take 100 μL MTT (5×), add to 400 μL MTT Diluent Buffer, and the mixture is 1× MTT working solution.
   Note: Prepare the fresh solution before use. 1×MTT working solution should be stored in dark.

2. Add 100 μL of cell suspension to each well of the 96 well microplate, and set blank wells (no cells but add 100 μL of culture medium).
   Note: For cell proliferation test, add 100 μL (about 2,000 cells) cell suspension to each well. For cell cytotoxicity test, add 100 μL (about 5,000 cells) cell suspension to each well. The seeding cells in each well depends on the size of the cell and the rate of cell proliferation, etc.

3. Culture the cells according to the experimental design.

4. Add 50 μL of 1×MTT working solution to each well and incubate for 4 h.
   Note: MTT incubation conditions are the same as cell culture conditions.

5. Discard the supernatant carefully, add 150 μL DMSO (self-prepared) to dissolve the formazan and shake the microplate with an incubation shaker.
   Note: To avoid discard the floating cells, please centrifuge first and then discard the supernatant.

6. Measure the OD value with microplate reader at 570 nm after the formazan was fully dissolved.

7. Calculation.

$$\text{Cell Survival Rate (\%)} = \frac{\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}}}{\text{OD}_{\text{control}} - \text{OD}_{\text{blank}}} \times 100\%$$

$$\text{Inhibition Rate} = \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}}{\text{OD}_{\text{control}} - \text{OD}_{\text{blank}}} \times 100\%$$

**[Note]:**

$OD_{sample}$: the OD value of sample well.

$OD_{control}$: the OD value of control well.

$OD_{blank}$: the OD value of blank well.

## Cautions

1. This kit is for research use only.
2. For your safety and health, please take safety precautions and follow the procedures of laboratory reagent operation. Wear laboratory clothes and disposable gloves during operation, and avoid direct contact with the human body or inhalation of the body.
3. For maximal assay performance, this reagent should be used within 6 months. Avoid freeze/thaw cycles.
4. MTT is yellow and needs to be stored in dark, otherwise it will lose its effectiveness. Do not use when the color turns grey green.
5. Pay attention to mixing during cell seeding to avoid unequal number of cells per well due to cell sedimentation.
6. The incubation time of MTT is generally 1~4 hours. It is recommended to take a preliminary experiment to explore the optimal number of cells and the incubation time of MTT.
7. When using a 96-well plate for cell culture, pay attention to the result error caused by water evaporation. It is recommended to discard the outer circle of wells and add PBS, water or culture medium to prevent water evaporation. In addition, the 96-well plate can also be placed in the incubator near the water.
8. Make sure that there is no bubble in each well when measuring the OD value with the microplate reader, otherwise it will interfere with the determination.
9. The detection of this kit relies on the dehydrogenase catalyzed reaction, so reducing agents (such as some antioxidants) will interfere with the detection. If there are reducing agents in the system, try to remove them. Or replace the fresh medium before adding MTT to remove the influence of the reagent.


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