TECHNICAL MANUAL

MTT Cell Proliferation and Cytotoxicity Assay Kit

- **SKU CODE:** AKES077
- **SIZE:** 500 Assays
- **DETECTION PRINCIPLE:** Apoptosis Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Kit components & storage

Cat.	Products	500 Assays	1000 Assays	Storage
	MTT (5×)	5 mL×1	5 mL×2	2~8°C, protect from light
	MTT Diluent Buffer	12.5 mL×2	12.5 mL×4	2~8°C
	Formazan Dissolution Buffer	50 mL	50 mL×2	2~8°C, protect from light
	Manual	One Copy		

2. Storage

Store at 2~8°C for one year. MTT (5×) should be stored in the dark.

3. Introduction

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

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

4. Staining Procedure

Method 1: For suspension cells and adherent cells

5. Reagent Preparation

MTT (5×) is concentrated and must be 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 becomes 1× MTT working solution.

Note: Prepare fresh solution before use. 1× MTT working solution should be stored in the dark.

6. Procedure - Method 1

1. 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 tests, add 100 µL (about 2,000 cells) cell suspension to each well. For cell cytotoxicity tests, add 100 µL (about 5,000 cells) cell suspension to each well. The number of seeded cells in each well depends on the cell size and proliferation rate.
2. Culture the cells according to the experimental design.
3. Add 50 µL of 1× MTT working solution to each well and incubate for 4 hours. Note: MTT incubation conditions are the same as cell culture conditions.
4. Bring the Formazan Dissolution Buffer to room temperature in advance. Add 100 µL Formazan Dissolution Buffer to each well, then incubate for 1~4 hours so that the formazan can be fully dissolved (to promote formazan dissolution, incubate on an incubation shaker at 37°C).
5. Observe under microscope and measure the OD value with a microplate reader at 570 nm after the formazan is fully dissolved. Note: The maximum volume per well of a 96-well microplate should not exceed 300 µL. When performing MTT assay using Method 1, ensure that the total volume of cell suspension and drug treatment solution does not exceed 150 µL. If the total volume is relatively large, centrifuge to remove excess liquid first, then proceed with subsequent assay procedures.

7. Method 2: For adherent cells

1. **Reagent Preparation:** MTT (5×) is concentrated and must be 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 becomes 1× MTT working solution. Note: Prepare fresh solution before use. 1× MTT working solution should be stored in the 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 tests, add 100 µL (about 2,000 cells) cell suspension to each well. For cell

cytotoxicity tests, add 100 µL (about 5,000 cells) cell suspension to each well. The number of seeded cells in each well depends on the cell size and proliferation rate.

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 hours. Note: MTT incubation conditions are the same as cell culture conditions.
5. Carefully discard the supernatant, add 150 µL DMSO (self-prepared) to dissolve the formazan and shake the microplate with an incubation shaker. Note: To avoid discarding floating cells, centrifuge first and then discard the supernatant.
6. Measure the OD value with a microplate reader at 570 nm after the formazan is fully dissolved.

8. Calculation

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

Inhibition Rate = $(OD_{control} - OD_{sample}) / (OD_{control} - OD_{blank}) \times 100\%$

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

9. Cautions

1. This kit is for research use only.
2. For your safety and health, please take safety precautions and follow laboratory reagent operation procedures. Wear laboratory clothes and disposable gloves during operation, and avoid direct contact with skin or inhalation.
3. For maximal assay performance, this reagent should be used within 6 months. Avoid freeze/thaw cycles.
4. MTT is yellow and must be stored in the dark, otherwise it will lose effectiveness. Do not use when the color turns grey-green.
5. Pay attention to mixing during cell seeding to avoid unequal numbers of cells per well due to cell sedimentation.
6. The incubation time for MTT is generally 1~4 hours. It is recommended to perform a preliminary experiment to determine the optimal cell number and MTT incubation time.

7. When using a 96-well plate for cell culture, pay attention to result errors caused by water evaporation. It is recommended to discard the outer wells and add PBS, water, or culture medium to prevent water evaporation. Additionally, the 96-well plate can be placed near water in the incubator.

8. Ensure there are no bubbles in each well when measuring OD values with the microplate reader, as bubbles will interfere with the measurement.

9. The detection of this kit relies on dehydrogenase-catalyzed reactions, so reducing agents (such as some antioxidants) will interfere with detection. If reducing agents are present in the system, try to remove them or replace with fresh medium before adding MTT to eliminate reagent interference.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.