

Produktinformation



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(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !)

MTT Assay Kit (Cell Proliferation)

Catalog No: E-CK-A341 Size: 500 Assays / 1000 Assays

Cat.	Products	500 Assays	1000 Assays	Storage
E-CK-A341A	MTT(5 ×)	5 mL	10 mL	2~8°C
E-CK-A341B	MTT Substrate	12.5 mL× 2	10 mL× 5	2~8°C
Manual		One Copy		

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA) Email: <u>techsupport@elabscience.com</u> Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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Introduction

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

Detection Principle

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 DMSO 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 cells, there is a linear relationship between the depth darkness of color and the number of cells.

Reagents Not Included

DMSO, PBS

Instructions

MTT(5 \times)[E-CK-A341A] is concentrated, please diluted with MTT Substrate to 1 \times MTT working solution before use.

For example: take 100 μ L MTT(5 ×)[E-CK-A341A], add to 400 μ L MTT Substrate[E-CK-A341B], and the mixture is 1 × MTT working solution.

Experimental Procedure

- Add 100 μL(about 2,000 cells) of cell suspension per well, incubate at 37 °C, 5% CO₂ Cell incubator for 24 h. Tips: A. For cell proliferation test, add 100 μL(about 2,000 cells) to each well, for cell cytotoxicity test, add 100 μL (about 5,000 cells) to each well(The number of cells used in each pore depends on the size of the cell and the rate of cell proliferation, etc.). B. The incubate temperature is different depending on cell types, most mammalian cells are recommended at 37 °C Other species please choose the appropriate temperature according to cell culture conditions.
- 2. Add 10 µL specific drug stimulation to each well to stimulate the cells (Two blank wells were added with the same volume of PBS without drugs).
- 3. Incubate at 37 ℃, 5% CO₂ and 100% humidity Cell incubator for appropriate time. Tip: Ditto. Selection of appropriate incubation conditions and time according to different cell type.
- Add 50 μL1 × MTT working solution to each well, incubate at 37 °C, 5% CO₂ Cell incubator for 4 h. Tip: Ditto. Selection of appropriate incubation conditions and time according to different cell type.
- 5. Remove the supernatant carefully, add 150 µL DMSO to each to dissolve the formazan format on the last step, shake the well with a flat shaker.
- 6. Measure the absorbance at 570 nm, if there is no 570 nm filter, the 560 nm~600 nm filter can be used.
- 7. Result Analysis

A. Cell Survival Rate. The OD value of each test well was subtracted from the background OD value (blank group, without drug stimulation), and the OD value of each repetitive well was taken as an average (\pm SD).

Cell Survival Rate % =(drug stimulation cells OD/blank group cells OD)×100%.

B. Calculated when T/C = 50%, the dug stimulation concentration (IC₅₀) and T/C = 10% the dug stimulation concentration (IC₉₀).

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Storage

Store at $2 \sim 8 \,^{\circ}$ C for half a year, MTT (5 ×) [E-CK-A341A] should be stored in dark.

Cautions

- 1. For maximal assay performance, this reagent should be used within 6 months. Avoid freeze / thaw cycles.
- 2. Suspension cells must be centrifuged and absorb supernatant before adding DMSO.
- 3. The format of formazan is depend on living cells and incubate time, Mix the cell suspension before adding to the wells to avoid cell precipitation leading to different cell number in each well. The OD value may change rapidly with time when the cell number is large.
- 4. MTT is yellow and needs to be stored in dark. Long-term illumination will lead to failure. Do not use when the color turns grey green. MTT may solidify at 4 °C or lower temperature. It can be incubated in water bath at 20~25 °C for a short time until it is completely melted before use.
- 5. For your safety and health, please wear the lab coat and disposable gloves before the experiments.