XTT Cell Proliferation Assay Kit

Introduction

XTT Cell Proliferation Assay Kit is a widely used cell proliferation and toxicity assay kit. XTT, a novel tetrazolium salt, can be reduced by succinate dehydrogenase in the mitochondria of living cells to the water-soluble formazan crystals. The amount of formazan can be quantified by measuring the absorbance at 450 nm upon solubilization and is proportional to the number of living cells.

Compared with the widely used MTT Assay Kit, this kit does not need to remove the culture medium, nor to use a solvent to dissolve the formazan, which is more convenient to use, and can avoid the experimental error caused by taking away part of the formazan when removing the medium. The kit is very flexible and can be continuedly incubated after detection for better color rendering. At the same time, this kit is safe for use, highly sensitive and has a good linear range.

Components and Storage

Size / Components	500 Tests	1000 Tests	Storage
XTT Solution	25 mL	2×25 mL	-20°C away from light
Electron Coupling Reagent (50×)	500 µL	1 mL	-20°C away from light
Shipping: Blue ice	Shelf life: 1 year		

Protocol

1. **The XTT working solution preparation:** Mix XTT Solution and Electron Coupling Reagent (50×) in a ratio of 50:1 to make an XTT working solution. Prepare a fresh XTT working solution every time.

***Note:** Before use, thaw the XTT Solution in a 37ºC water bath, and thaw the PMS Solution at room temperature. After thawing, vortex for a few moments respectively to ensure that the solution is completely mixed.

2. **Cell culture:** For 96-well plates, seed cells at a density of 5-100x10^3 cells/well in 100 µL 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.

3. **XTT incubation:** Add 50 µL XTT working solution per well, and incubate at 37°C for 3-4 h.

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

4. **Detection:** Measure the absorbance (A) at 450 nm with a microplate reader.

5. **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

1. 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.

2. Reducing agents, such as some antioxidants, can interfere with detection. When detecting these reagents, change the fresh medium before adding the XTT working solution to remove interference.

3. For your safety and health, please wear lab coats and gloves during the experiment.

4. For research use only. Not to be used in clinical diagnostic or clinical trials.

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