XTT Cell Proliferation and Cytotoxicity Assay Kit

C1505852

Storage -20℃. Store in the dark.

Introduction

This product is a rapid and highly sensitive detection kit based on XTT [2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazole-5-formamide inner salt], which is widely used for cell proliferation and cytotoxicity analysis. When XTT is present in an electron-coupling reagent, it is reduced by the reducing dehydrogenase produced within living cells to a water-soluble orange-yellow metachrome product. This can be detected by an enzyme reader at a wavelength of 450 nm. The stronger the cell viability, the more metachrome is generated, and the darker the color. Within a certain range, the absorbance is proportional to cell viability. Therefore, cell proliferation and cytotoxicity analysis can be conducted using XTT.

This product is simple to operate. Just mix the XTT Labeling Reagent and the Electron-coupling Reagent into a working solution and add it directly to the cells. Incubate at 37°C for a certain period of time. There is no need to collect the cells or wash them. Just use the enzyme reader to measure the absorbance at a wavelength of 450 nm directly.

This product can be widely used for cell proliferation determination (such as studies on cell induction proliferation by growth factors, cytokines, etc.) and cytotoxicity determination (such as studies on the cytotoxicity of anti-cancer drugs).

Component

C1505852	Component	500T	1000T	Storage
C1505852A	XTT Labeling Reagent	25 mL	50 mL	-20℃. Store in the dark.
C1505852B	Electron-coupling Reagent	0.5 mL	1 mL	-20℃. Store in the dark.

Note: The 500T package of this kit can test 500 samples; the 1000T package can test 1000 samples.

Preparation before the experiment

1. Reagents & Consumables

PBS、96-well cell culture plates (or other cell culture plates) for absorbance measurement, cells.

2. Instruments

Microplate reader (with 450 nm filter), CO_2 incubator, etc.

Usage method

1. Preparation of XTT working solution:

Mix XTT Labeling Reagent and Electron-coupling Reagent in a ratio of 50:1 to prepare the XTT working solution. Place the prepared solution on ice for later use (use immediately after preparation).

2. Standard Curve Preparation (Optional):

The cells were inoculated into 96-well cell culture plates at certain gradients (for example, each well contained 100 μL of culture medium with 0, 2000, 4000, 8000, 16000, 32000, and 64000 cells respectively). Generally, 5 to 8 cell quantity gradients were set up, and 3 to 6 replicates were conducted for each group.

3. Cell culture:

a Select cells in a healthy growth state, adjust the cell density, and inoculate 100 μL of cell suspension per well into a 96-well plate. At the same time, set a blank well (without adding cells but adding the same volume of culture medium);

b Place it in the cell culture box (at 37℃, 5% CO_2) for 24-48 hours of cultivation;

c According to the experimental design, cultivate and treat the cells.

4. Cell Viability Assay:

a Add 50 μL of the XTT working solution to each well and mix well;

b Place in the cell culture box (37°C, 5% CO_2) and incubate for 2-4 hours;

Note: The incubation time varies depending on the independent experimental setup (such as the type of cells used and the cell concentration). Therefore, we recommend following the described method to measure the absorbance using an enzyme reader every 1 hour after adding the XTT working solution, and select the appropriate time point with suitable absorbance values for the subsequent experiments.

5. Detection:

Measure the absorbance values of each well at 450 nm. If there is no 450 nm filter, a 450-490 nm filter can be used.

6. Cell vitality calculation:

Cell viability (%) = (A - C) / (B - C) × 100

Inhibition rate (%) = (B - A) / (B - C) × 100

A represents the absorbance of the experimental group: the experimental group contains cells treated with a specific drug, culture medium, and XTT detection solution.

B represents the absorbance of the control group: the control group contains cells without

drug treatment, culture medium, and XTT detection solution.

C represents the absorbance of the blank group: the blank group contains no cells, but contains culture medium and XTT detection solution.

Matters needing attention

1. The XTT Reagent and Electron Coupling Reagent should be stored in a dark place. During the experiment, be sure to operate in a dark environment.
2. Before conducting the formal experiment, select several samples for a preliminary test to optimize the experimental conditions and determine the optimal cell dosage for achieving the best experimental results.
3. The reagents should not be repeatedly frozen and thawed. When thawing for dissolution, make sure to remix thoroughly.
4. Since the detection is carried out using a 96-well plate, if the cell culture time is long, be sure to pay attention to the issue of evaporation. You can adopt the method of discarding the surrounding area and replacing it with PBS; or place the 96-well plate near the water source in the incubator to alleviate evaporation. When inoculating, make sure to mix the cell suspension thoroughly to avoid cell precipitation, which may result in unequal cell counts in each well. You can mix the suspension after inoculating several wells.
5. When inoculating, pay attention to thoroughly mixing the cell suspension to avoid cell precipitation, which may cause unequal cell counts in each well. Mix the suspension after inoculating several wells.
6. Before using the spectrophotometer for detection, ensure that there are no bubbles in the wells. Otherwise, it will interfere with the measurement.
7. Contamination of samples or reagents by bacteria or fungi or cross-contamination of reagents may lead to incorrect results.
8. For your safety and health, please wear a lab coat and wear disposable gloves.