Product Name: Dihydroethidium (Hydroethidine; DHE)

Product number: RA20030

Basic Information

Product name	Dihydroethidium (Hydroethidine;DHE)
Size	1 mL
Storage	-20℃, protected from light
Shipping	Shipped with ice pack
Validity	12 months

Product Introduction

DHE (Dihydroethidium) can freely penetrate the living cell membrane into the cell and be oxidized by ROS in the cell to form ethidium oxide; ethidium oxide can be incorporated into chromosomal DNA to produce red fluorescence. The amount and changes of cellular ROS content can be determined based on the generation of red fluorescence in living cells. DHE is mainly oxidized by superoxide anion ROS in cells and can be directly observed using a flow cytometer or fluorescence microscope. It is a quick and easy classic method for detecting ROS in tissues or cultured living cells.

Experimental procedures

1. Staining method:

(1) The probe solution can be diluted to the desired concentration in fresh culture medium, buffered saline solution or tissue perfusion fluid, and the cell culture medium or perfusion fluid can be replaced with this staining solution; or the probe can be directly added to the cell incubation fluid or perfusion fluid to the desired concentration.

(2) Depending on the ROS content of the cells, the final concentration of DHE can be selected in the range of 1 μM~100 μM, and the incubation time can be selected from 10 to 90 min. The incubation can be carried out at 37°C or room temperature and should be protected from light.

(3) After incubation, wash the cells or tissues with fresh solution.

2. Fluorescence microscopy operation method:

(1) For adherent cells or living tissues, they can be observed directly under a fluorescence microscope. For suspended cells, drop 25-50 μL of the cell suspension onto a microscope slide and cover it with a coverslip.

(2) Under a fluorescence microscope, the red emission images of cells are observed and photographed using blue or green light for excitation. ROS-positive cells are stained red in the entire nuclear region. When excited by ultraviolet light, unoxidized DHE in the cytoplasm can emit blue fluorescence.

3. Flow cytometry analysis method:

(1) For adherent cells, digest with trypsin to prepare a single-cell suspension; for suspended cells, collect the cells directly. Resuspend the cells (50,000 to 100,000) in 0.5-1 mL ice-cold PBS.

(2) Using an excitation wavelength of 480-535 nm and measuring the emission above 590 nm-610 nm, the cells should be divided into two subpopulations: ROS-negative cells have only very low fluorescence intensity, and ROS-positive cells have strong red fluorescence.

Unoxidized state excitation/emission wavelength: 355/420 nm
Excitation/emission wavelength after oxidation: 518/610 nm

Precautions

1. DHE is easily oxidized in light and air, so keep it away from light.
2. To avoid repeated freezing and thawing, this product can be divided into small quantities.
3. This reagent can be used for testing living cells cultured in vitro, cultured or perfused tissues, and frozen

tissue sections.
4. For different cells and tissues, appropriate incubation time and concentration should be selected to observe the changes in ROS.

Note: This reagent is for scientific research use only!