

Product Information

ROS-DHE

Catalog Number: D1008

Product Size: 1 mL (5 mM in DMSO)

Parameters

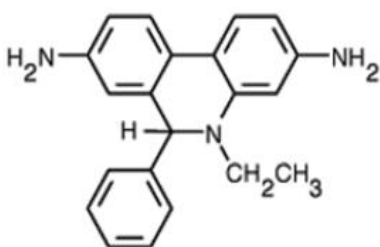
Ex/Em: 355/420 nm

CAS No.: 38483-26-0

Molecular Formula: C₂₁H₂₁N₃

Molecular Weight: 315

Molecular Structure:



Storage

Store at -20°C and protect from light. When stored as directed, product is stable for at least 6 months.

Description

The superoxide indicator dihydroethidium, exhibits blue-fluorescence in the cytosol until oxidized, where it intercalates within the cell's DNA, staining its nucleus a bright fluorescent red. According to the production of red fluorescence in living cells, the amount and change of ROS content in cells can be detected. Dihydroethidium is mainly oxidized by superoxide anion ROS in the cell. It can be directly observed by flow cytometry or fluorescence microscope. It is a fast and simple classic method for detecting ROS in living tissue or cultured cells.

Protocol

1. Staining method:

1) The probe solution can be diluted to the required concentration in fresh culture solution, buffer solution or tissue perfusate, and the cell culture solution or perfusate can be replaced by this staining solution. The probe solution may also be added directly to the cell incubation or perfusion solution to the desired concentration.

2) Depending on the ROS content of the cells, the final concentration of dihydroethidium can be selected from the range of 1 μM to 100 μM, and the incubation time can be selected from 10 to 90 min. Incubation can be performed at 37 °C or room temperature, requiring protection from light.

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

2. Fluorescence microscope operation method:

1) For adherent cells or living tissues, you can observe directly under a fluorescent microscope; for suspended cells, 25-50 μL cell suspension is dropped on a microslide, and then covered with a cover slide.

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

3. Flow cytometry operation method:

1) For adherent cells, trypsin digestion is used to prepare a single cell suspension; for suspended cells, cells (50,000 to 100,000) are directly collected and resuspended with 0.5 to 1





mL cold PBS.

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

Notes

1. Dihydroethidium is easily oxidized in the light and air. Please keep it away from light.
2. The reagent can be used for the detection of cultured live cells in vitro, cultured or perfused tissues and frozen sections of tissues.
3. For different cells and tissues, the appropriate incubation time and concentration should be selected to observe change of ROS.

