

## Dihydrorhodamine 123

Cat: ID3720

**Storage:** Powder: -20°C, 2 years; Insolvent : -20°C, 6 months; -80°C, 1 year (protect from light)

### Introduction

Dihydrorhodamine 123 (DHR 123) is the reduced form of rhodamine 123, a commonly used fluorescent mitochondrial dye. Dihydrorhodamine 123 itself is non-fluorescent, but it can easily penetrate cell membranes to enter cells and be oxidized by intracellular oxidizing substances or redox systems to fluorescent rhodamine 123, which accumulates on the mitochondrial membrane and emits bright green fluorescence. Nitric oxide (NO), superoxide, or hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) alone cannot oxidize dihydrorhodamine 123, but these reactive oxygen species can oxidize DHR 123 to produce rhodamine 123 when combined with other cellular components, such as cytochrome c oxidase or Fe<sup>2+</sup>. The fluorescent signal can be detected with a fluorometer, flow cytometer, or fluorescence microscope.

Dihydrorhodamine 123 can be used to detect reactive oxygen species (ROS), including superoxide (in the presence of peroxidase or cytochrome c) and peroxynitrite. Dihydrorhodamine 123 is widely used in a variety of cells, such as human neutrophils, lung tumor SPC-A-1 cells, endothelial cells, HaCaT cells, eosinophils, murine mast cells, guinea pig neutrophils, chondrocytes, and murine proximal renal tubule cells.

### Parameter

Ex/Em: 488/525nm

CAS: 109244-58-8

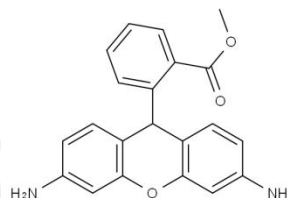
Molecular Formula: C<sub>21</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub>

Molecular Weight: 346.38

Purity: HPLC≥95%

Appearance: Solid

Solubility: Soluble in DMSO(Need ultrasonic)



### Protocols (only for reference)

#### Preparation of storage solution

Prepare a 5 mM stock solution in DMSO. For example, 1.7 mg of DHR123 powder was dissolved in 982 μL of DMSO.

Note:

- Unused storage solution is recommended to be stored in portions at -20°C to avoid repeated freezing and thawing.
- Moisture-absorbing DMSO has a significant effect on the solubility of the product, use freshly opened DMSO.

#### Preparation of working fluid

Dilute the reservoir solution with a suitable buffer (e.g. serum-free medium or PBS, etc.) to make a 5  $\mu\text{M}$  DHR 123 working solution.

Note:

- a. The final concentration of the working solution is recommended to be optimized according to different cell lines and experimental systems.
- b. If it is found difficult to dissolve, it can be sonicated to promote dissolution.
- c. Please adjust the concentration of the working solution according to the actual situation, and use it now.

### Staining of adherent cells

- 1) Select the appropriate cell density to inoculate the adherent cells and incubate them overnight.
- 2) The drug of interest can be selected to intervene in the cells and continue to culture them for a certain period of time.
- 3) The cell culture was aspirated and stained by adding DHR 123 working solution.
- 4) Incubate at 37°C in a cell culture incubator for 15-60 minutes.
- 5) The staining solution was aspirated, and the cells were washed with PBS and observed by fluorescence microscopy.

### Staining of suspension cells

- 1) Cultivate a sufficient amount of suspension cells ( $1 \times 10^6$  cells/ mL).
- 2) If drug stimulation is to be performed, intervene by adding the drug of interest to the cells and continue culturing for a certain period of time.
- 3) Collect by centrifugation and wash the cells with PBS.
- 4) The cells were resuspended by adding DHR 123 Working Solution and incubated at 37°C for 15-60 min, protected from light.
- 5) It was detected by flow cytometry, or a cell suspension can be taken in slide form and examined under a fluorescence microscope.

### Note

1. Due to the different cell types and experimental systems, the concentration of DHR 123 working solution and incubation time can be adjusted according to the pre-tests or references.
2. All fluorescent dyes have quenching problems, please try to avoid light to slow down the fluorescence quenching.
3. For your safety and health, please wear lab coat and disposable gloves.
4. This product is for scientific research use only. Do not use in medicine, clinical diagnosis or treatment, food and cosmetics. Do not store in ordinary residential areas.