MitoMark Red I

Cat: IF1770

Storage: Powder: -20℃, 2 years; Insolvent (mother liquid): -20℃, 6 months; -80℃, 1 year (rotect from light)

Introduction

Mitochondrial Red I is a red fluorescent dye containing chloromethyl functional groups that labels mitochondria. The Mitochondrial Red I can be transported passively across cell membranes and directly onto active mitochondria by simple incubation with living cells, and the accumulation of the dye is dependent on the membrane potential. Once the mitochondria have been stained, they can also be fixed with aldehyde fixatives as required for subsequent experiments. For immunohistochemistry and in situ hybridization experiments where cells are permeabilized, the Mitochondrial Red I also stains the mitochondria of permeabilized cells. The dye is suitable for double labeling experiments, and its red fluorescence can be well distinguished from other green fluorescent probes.

Although conventional mitochondrial fluorescent probes, such as TMR and rhodamine 123, can also be readily aggregated on functional mitochondria, they are washed out once the mitochondrial membrane potential is lost, which limits their use in experiments that require cellular aldehyde immobilization or that include factors that influence mitochondrial energy status.

Parameter

Ex/Em: 579/599 nm

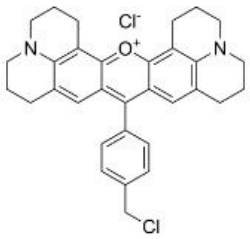
CAS: 167095-09-2

Molecular Formula: $C_{32}H_{32}Cl_2N_2O$

Molecular Weight: 531.52

Appearance: Brown to black Soild

Solubility: Soluble in DMSO



Protocols (*only for reference*)

Preparation of storage solution

Prepare a 1 mM stock solution in DMSO. e.g. 50 μg of powder dissolved in 94 μL of DMSO.

Note:

a. Unused storage solution is recommended to be stored in portions at -20℃ to avoid repeated freezing and thawing.

b. Moisture-absorbing DMSO has a significant effect on the solubility of the product, please use freshly opened DMSO.

c. It is recommended to return the product to room temperature before use.

Preparation of working fluid

Dilute the reservoir with a suitable buffer or cell culture medium to formulate a working solution of 25-500 nM.

Note:

a. The final concentration of the working solution is recommended to be optimized according to different cell lines and experimental systems.
b. For samples that require subsequent fixation or permeabilization, a working concentration of 100-500 nM is recommended.
c. In order to reduce potential artifacts and mitochondrial toxicity caused by overloading, staining should be done at as low a concentration as possible without affecting the experimental results.
d. High concentrations may also stain other cellular structures.

Stain

Adherent cells

1) Add appropriate amount of culture medium to the petri dish/plate to cover the coverslip for crawling culture.
2) When the cells grow to the desired abundance, aspirate the culture medium and add the mitochondrial red fluorescent probe staining working solution preheated at 37°C.
3) Incubate for 15-45min under normal culture conditions of the cells used (optimal incubation time needs to be optimized).
4) At the end of staining, replace the above staining solution with fresh culture medium or buffer, and it can be placed under a fluorescence microscope for observation or a fluorescence enzyme marker for reading.

Suspension cells

1) Collect the cells by centrifugation, discard the supernatant, and gently resuspend the cells using pre-warmed mitochondrial red fluorescent probe staining working solution at 37°C.
2) Incubate for 15-45 min under normal culture conditions of the cells used (optimal incubation time to be optimized).
3) At the end of staining, collect the cells by centrifugation and resuspend the cells using fresh culture medium or buffer pre-warmed at 37°C.
4) Stained cells can be analyzed by flow cytometry, fluorescence enzyme marker, and fluorescence microscope.

Note: If immobilized cells on coverslips are required, then slides or coverslips can be coated with poly-D-lysine prior to laying.

Fixation and permeabilization (*optional*)

1) After staining, wash the cells using culture solution or buffer.
2) Carefully aspirate the wash solution. Replace with freshly prepared and pre-warmed buffer or culture solution containing 2-4% formaldehyde for cell fixation.
3) Wash the cells: Aspirate off the fixative and rinse the cells several times with appropriate buffer.
4) Cell permeabilization (optional):

For experiments such as ICC that require cells to be permeabilized, the fixed cells can be

incubated directly in buffer containing a detergent such as Triton X-100. After permeabilization, the cells can be washed with the buffer for subsequent ICC experiments.

Alternatively, pre-cooled acetone can be used for permeabilization for 5 min, after which the cells can be washed with PBS. Acetone permeabilization has been shown to reduce the background signal even in the absence of further antibody labeling.

Note

1. Please centrifuge the product instantaneously to the bottom of the tube before use and then proceed with subsequent experiments.
2. For different cells and tissues, the appropriate incubation time and staining solution concentration should be selected.
3. All fluorescent dyes have quenching problems, please try to avoid light to slow down the fluorescence quenching.
4. For your safety and health, please wear lab coat and disposable gloves.
5. This product is for scientific research use only. Do not use in medicine, clinical diagnosis or treatment, food and cosmetics. Please do not store in ordinary residential areas.

Related Products

IF1780 Mitochondrial green fluorescent probes
IJ0300 JC-1
IJ0310 JC-10