Product Name: Mitochondrial Tracker (Red)

Catalog No.: RA20020

Basic Information

Product name	Mitochondrial Tracker (Red)
Size	50 μg/20 x 50 μg
Storage	-20℃, keep away from light
Shipping	Shipped with ice pack
Validity	12 months
Ex/Em	579/599 nm

Product Introduction

Mitochondrial Tracker (Red) is a red fluorescent dye containing a chloromethyl functional group, which can be used to label mitochondria. Mitochondrial Tracker (Red) can passively diffuse across the cell membrane and directly accumulate in active mitochondria simply by incubation with live cells. The accumulation of this dye depends on the membrane potential. Once the mitochondria are stained, they can be fixed with aldehyde fixatives according to the requirements of subsequent experiments. For experiments such as immunohistochemistry and in situ hybridization, cells need to be permeabilized first, and Mitochondrial Tracker (Red) can also stain the mitochondria of permeabilized cells. This dye is suitable for double-labeling experiments, as its red fluorescence can be well distinguished from other green fluorescent probes. Although traditional mitochondrial fluorescent probes such as TMR and rhodamine 123 can also easily accumulate in functional mitochondria, they will be washed away once the mitochondrial membrane potential is lost. This limits their application in experiments that require aldehyde fixation of cells or involve factors affecting mitochondrial energy status.

Reagent Preparation

Preparation of stock solution

Dissolve in high-purity anhydrous DMSO and prepare a stock solution with a concentration of 1 mM . The molecular weight of the dye is 531.5 g/mol . Simply adding 94 μL of DMSO to 50 μg of Mitochondrial Tracker (Red) obtain a 1 mM stock solution of the powder.

Note: ① Before opening the reagent tube, the product needs to be returned to room temperature.

② The storage solution can be divided into small tubes according to the single usage amount and stored at -20℃ away from light to avoid repeated freezing and thawing.

Preparation of working fluid

The dye concentration varies according to different cells and experimental requirements. The following operating conditions are for reference only and can be adjusted according to cell types and other

relevant factors such as such as permeabilization of cells or tissues.
Dilute the 1 mM stock solution to the working concentration with an appropriate buffer (such as PBS) or cell culture medium. A general recommended concentration range is 25-500 nM.
For samples that require fixation or permeabilization in subsequent steps, a working concentration of 100-500 nM is recommended. To minimize potential artifacts and mitochondrial toxicity caused by overloading, the staining solution concentration should be reduced as much as possible without affecting the experimental results. Additionally, higher concentrations may also stain other cellular structures.
Note: Since oxidases in the culture medium can weaken the effect of the dye, it is not recommended to use complete culture medium to dilute the stock solution in this protocol.

Operation Steps

Adherent cell staining

(1) Add an appropriate amount of culture medium to the culture dish/plate to cover the cells and culture the cells . When the number of cells reaches a sufficient level, remove the culture medium.
(2) Add the staining working solution preheated at 37°C and incubate in a cell culture incubator for 15-45 min (adjust the incubation time according to the experiment).
(3) After staining, remove the staining solution and add fresh culture medium or buffer preheated at 37°C. Observe under a fluorescence microscope or obtain the result under a fluorescence microplate reader. Or proceed to subsequent fixation and permeabilization steps.

Suspension cell staining

(1) Collect cells by centrifugation and discard the supernatant.
(2) Add 37°C preheated staining working solution and gently pipette to resuspend the cells. Incubate in a cell culture incubator for 15-45 min (adjust the incubation time according to the experiment).
(3) After staining, collect the cells by centrifugation, add fresh culture medium or buffer preheated at 37°C to resuspend the cells, and analyze with a flow cytometer, microplate reader or fluorescent reader. If fixed cells on coverslips are required, poly-D-lysine can be used to coat the coverslips before spreading. Slides or coverslips. If fixation and permeabilization are required after staining , subsequent fixation and permeabilization steps can be performed.

Cell fixation (optional)

(1) After staining, wash the cells twice with culture medium or buffer.
(2) Fix the cells with freshly prepared and preheated buffer or culture medium containing 2-4% formaldehyde.Incubating endothelial cells in culture medium at 37°C for 15 min can achieve a good

fixation effect .
(3) Aspirate the fixative and wash the cells twice with appropriate buffer.

Cell permeabilization (optional)

For experiments requiring cell permeabilization, fixed cells can be directly incubated in a buffer containing a detergent such as Triton X-100. After permeabilization is complete, wash the cells twice with buffer, and the cells are ready for subsequent experiments. It has been tested that incubating endothelial cells in PBS containing 0.2% Triton X-100 for 10 minutes can achieve good permeabilization effects.
Alternatively, cells can be permeabilized with pre-cooled acetone for 5 minutes, followed by washing with PBS. It has been verified that even without further antibody labeling in subsequent steps, acetone permeabilization can reduce background signals.

Note

1. All fluorescent dyes have quenching problems. Please try to avoid light to slow down fluorescence quenching.
2. To avoid repeated freezing and thawing, this product can be divided into small quantities.
3. This product is For Research Use Only, Not for Diagnostic Use.