Product Information

MitoScene™ Red CMXRos

Catalog Number: M4067

Product Size: 50 μg / 20 × 50 μg

Application Scope: Mitochondrial dye

Parameters

Appearance: Purple solid soluble in DMSO

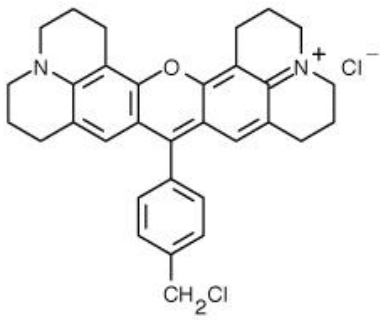
Ex/Em: 579/599 nm (in MeOH)

CAS NO.: 167095-09-2

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

Molecular Weight: 531.52

Molecular Structure:



Storage

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

Description

MitoScene™ Red CMXRos is a red fluorescent dye that contains chloromethyl functional groups to label mitochondria. The dye is membrane permeable and becomes brightly fluorescent upon accumulation in the mitochondrial membrane. Staining is dependent on mitochondrial membrane potential, and can be used to monitor mitochondrial membrane potential in intact cells. After staining, the sample can also be fixed with an aldehyde fixing agent according to the requirements of subsequent experiments. For immunohistochemistry and in situ hybridization experiments, cells need to be permeabilized first. MitoScene™ Red CMXRos can also stain mitochondria of permeabilized cells. The dye is suitable for double labeling experiments, and its red fluorescence can be distinguished from other green fluorescent probes well.

Traditional mitochondrial fluorescent probes such as Rhodamine 123 can also be easily clustered on functional mitochondria, but once the mitochondrial membrane potential is lost, it will be washed away. Therefore, in some experiments that require cells to fix aldehydes or contain mitochondrial energy state affecting factors, their applications are greatly restricted.

Protocol

1. Dyeing liquid preparation

Before opening the reagent, warm the product to room temperature.

(1) Preparation of stock solution: Dissolve one 50 μg vial of lyophilized dye in 94 μL anhydrous DMSO to prepare 1 mM stock solution.

Note: It is recommended to store the storage solution at -20 ℃, and aliquot it in small quantities to avoid repeated freeze-thaw cycles.

(2) Preparation of working solution: Dilute the stock solution with medium or PBS to prepare a working solution with a concentration of 25-500 nM. For subsequent samples that need

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to be fixed or permeabilized, the recommended working concentration is 100-500 nM. In order to reduce potential artifacts and mitochondrial toxicity caused by excessive loading, the concentration of staining solution should be reduced as much as possible without affecting the experimental results. In addition, too high a concentration may stain other cellular structures.

Note: You may need to optimize the staining procedure for each particular cell type by varying the dye concentration, staining volume, labeling time, or wash steps.

Note: The oxidase in the medium will weaken the effect of the dye. It is not recommended to use complete medium to dilute the stock solution in this operation.

2. Staining Cells

Adherent cell staining

(1) Remove growth medium from the cells.

(2) Add enough working solution to completely cover the cells.

(3) Incubate the cells at 37°C for 15-45 min. The optimal incubation time will vary depending on the cell type. Start with 5 minutes and optimize as needed for uniform labeling.

(4) Remove the working solution.

(5) Wash the cells by adding fresh warm growth medium or PBS. Repeat this wash step two more times.

(6) Image fluorescence or perform subsequent fixation and permeabilization steps.

Suspension cell staining

(1) Collect cells by centrifugation.

(2) Suspend cells at a density of 1×10^6/mL in working solution.

(3) Incubate for 15~45 minutes at 37°C. The optimal incubation time will vary depending on cell type. Start with 5 minutes and optimize as needed for uniform labeling.

(3) Pellet the cells by centrifugation at 1000～1500 rpm for 5 minutes.

(4) Remove the supernatant and wash the cells by gently resuspending them in warm (37°C) medium or PBS.

(5) Repeat the centrifugation and wash steps (Steps 3 and 4) two more times.

(6) Image fluorescence or perform subsequent fixation and permeabilization steps.

3. Cell fixation (optional)

(1) After staining, wash the cells twice with culture or buffer.

(2) Fix with 3.7% paraformaldehyde in PBS for 10-30 min at room temperature.

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

4. Cell permeabilization (optional)

(1) After staining, wash the cells twice with culture or buffer.

(2) Permeabilize with 0.2% Triton X-100 in PBS for 10-20 min. You also can be permeabilized with pre-cooled acetone for 5 min.

(3) Aspirate the permeabilizing solution and wash the cells twice with an appropriate buffer.

Notes

1. There are quenching problems with fluorescent dyes. Please avoid light to slow down the fluorescence quenching.

2. For your safety and health, please wear lab coats and disposable gloves.

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