Mito-Tracker Red CMXRos

Introduction

Mito-Tracker Red CMXRos, also called MitoMark Red I, is an oxidized red fluorescent probe that selectively labels mitochondria. Some conventional fluorescent probes for mitochondria such as tetramethylrosamine and rhodamine 123, are sequestered by functional mitochondria. These probes are easily washed out of cells upon loss in the mitochondria membrane potential. This characteristic limits the use in experiments that require cells to be treated with fixatives or other regent that affect the function of the mitochondria. Mito-Tracker Red CMXRos is a derivative of X-rosamine, which passively diffuses across the plasma membrane of live cells and accumulates in active mitochondria. Meanwhile, Mito-Tracker Red CMXRos is retained in the mitochondria if the cells need fixation after the mitochondria staining.

Components and Storage

Components	B8810-50 μg
Mito-Tracker Red CMXRos	50 μg

This product should be stored at -20°C away from light and moisture. Stable for 1 year.

Properties

Physical Appearance	Solid
M.Wt	531.52
Cas No.	167095-09-2
Formula	$C_{32}H_{32}Cl_2N_2O$
Ex/Em	578/598
Synonyms	MitoMark Red I
Solubility	Soluble in DMSO

Protocol

1. **Preparation of the stock solution:** Dissolve 50 μg Mito-Tracker Red CMXRos in 94 μL anhydrous DMSO to make a 1 mM stock solution. The stock solution should be stored at -20°C away from light. It is recommended to aliquot the stock solution into small volumes and avoid repeated freeze/thaw cycles.

***Note:** Allow Mito-Tracker Red CMXRos to warm to room temperature before opening.

2. **Preparation of the working solution:** Dilute the stock solution in a suitable buffer (for example, HHBS) or growth medium to make a working solution. The recommended concentration of the working solution can be in the range of 100-500 nM. To reduce potential mitochondria toxicity and non-specific staining, keep the concentration of working solution as low as possible. It is suggested to dilute Mito-Tracker Red CMXRos when using it.

***Note:** The optimal concentration of working solution varies depending on the type of cells.

3. **Labeling of Mitochondria:** For adherent cells, grow cells to reach the desired density. Remove the growth medium and add a pre-warmed (37°C) working solution to cover the cells. Incubate at 37°C away from light for 15-45 min. Replace the working solution with a fresh, pre-warmed, suitable buffer or growth medium. Then detect the fluorescence signal of cells. If the cells need to be fixed and permeabilized, continue to perform step 4.

***Note:** The optimal time for incubation varies depending on the type of cells. For suspension cells, harvest cells and perform similarly to the adherent cells.

4. **Fixation and permeabilization (optional step):** After staining live cells with Mito-Tracker Red CMXRos, wash cells in a fresh, pre-warmed, suitable buffer. Remove the buffer and replace it with a fixative (for example, 2-4% formaldehyde in a suitable buffer). After fixation, wash cells gently in a fresh, pre-warmed, suitable buffer several times. If permeabilization is needed, incubate fixed cells in a suitable buffer containing permeabilization agent (for example, Triton X-100). After the permeabilization, wash cells in a fresh, pre-warmed, suitable buffer and proceed with the immunocytochemistry procedure.

Note

1. Fluorescent probes are easy to quench, please protect them from light when using.
2. For your safety and health, please wear lab coats and gloves during the experiment.
3. For research use only. Not to be used in clinical diagnostic or clinical trials.

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