MitoTracker®Red CMXRos Mitochondrial Probe

Cat. No. PA-3017

Storage upon receipt: -20°C

Avoid freeze-thaw cycles

Desiccate

Protect from light

Solvent for stock: DMSO

Molecular Weight: 531.52

Instructions for Use

Although conventional fluorescent stains for mitochondria, such as tetramethylrosamine and rhodamine 123, are readily sequestered by functioning mitochondria, these stains are easily washed out of cells once the mitochondria experience a loss in membrane potential. This characteristic limits the use of such conventional stains in experiments that require cells to be treated with aldehyde fixatives or with other agents that affect the energetic state of the mitochondria. MitoTracker Red is concentrated by active mitochondria and well retained during cell fixation.[1]

The cell-permeant MitoTracker probe contains a mildly thiolreactive chloromethyl moiety that appears to be responsible for keeping the dye associated with the mitochondria after fixation.

To label mitochondria, cells are simply incubated in submicromolar concentrations of a MitoTracker probe, which passively diffuses across the plasma membrane and accumulates in active mitochondria. Once their mitochondria are labeled, the cells can be treated with an aldehyde-based fixative to allow further processing of the sample. Because most of the MitoTracker probe is also retained after permeabilization with detergents or organic solvents, the sample continues to exhibit the fluorescent staining pattern characteristic of live cells during subsequent processing steps (e.g., immunocytochemistry, in situ hybridization or electron microscopy). In addition, MitoTracker eliminates some of the difficulties of working with pathogenic cells because, once the mitochondria are stained, the cells can be treated with fixatives before the sample is analyzed.

Storage and Handling

Upon receipt, the lyophilized solids should be stored desiccated at –20°C until required for use. When stored as solids, these reagents are stable for at least six months.

AVOID REPEATED FREEZING AND THAWING.

Before opening a vial, allow the product to warm to room temperature. To prepare a stock solution, dissolve the lyophilized product in high-quality, anhydrous dimethylsulfoxide (DMSO) to a final concentration of 1 mM. It is preferable to use solutions of the dihydro derivatives on the day that they are prepared. All other solutions of the MitoTrackerRed can be stored frozen at –20°C and protected from light.

Protocol

Cell Preparation and Staining

1.1 **Preparing staining solutions**. The concentration of probe for optimal staining will vary by application. The initial conditions suggested here are guidelines that may need to be modified based on the particular cell type or on other factors, such as the permeability of the cells or tissues to the probe. Dilute the 1 mM MitoTracker stock solution (see Storage and Handling for preparation) to the final working concentration in growth medium, e.g., Dulbecco's modified Eagle medium (D-MEM), with or without serum to match the medium that the cells were grown in. For live-cell staining, we recommend working concentrations of 25–500 nM. For staining cells that are to be fixed and permeabilized (see Fixation and Permeabilization after Staining), we suggest using a working concentration of 100–500 nM. To reduce potential artifacts from overloading, the concentration of dye should be kept as low as possible.

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Part No. 06868

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1.2 **Staining adherent cells**. Grow cells on coverslips inside a Petri dish filled with the appropriate culture medium. When cells have reached the desired confluence, remove the medium from the dish and add the prewarmed (37°C) growth medium containing the MitoTracker probe (prepared in step 1.1). Incubate the cells for 15-45 minutes under growth conditions appropriate for the particular cell type. Then replace the loading solution with fresh prewarmed medium and observe the cells using a fluorescence microscope fitted with the correct filter set (see Table 1). If the cells do not appear to be sufficiently stained, we recommend either increasing the labeling concentration or increasing the time allowed for the dye to accumulate in the mitochondria once the cells have been transferred to fresh medium. If the cells are to be fixed and permeabilized, continue to Fixation and Permeabilization after Staining.

1.3 Staining suspension cells. Centrifuge to obtain a cell pellet and aspirate the supernatant. Resuspend the cells gently in prewarmed (37°C) medium containing the MitoTracker probe (prepared in step 1.1). Incubate the cells for 15–45 minutes under growth conditions that are appropriate for the particular cell type. Re-pellet the cells by centrifugation and resuspend in fresh prewarmed medium. Again, if the cells are not sufficiently stained, we recommend increasing the labeling concentration or increasing the time allowed for the dye to accumulate in the mitochondria once the cells have been transferred to fresh medium. Alternatively, suspension cells may be attached to coverslips that have been treated with BD Cell-Tak™ Cell and Tissue Adhesive (BD Bioscience, Bedford, MA); in this case, see step 1.2. If the cells are to be fixed and permeabilized, continue to Fixation and Permeabilization after Staining.

1.4 Staining fixed cells. Following fixation, the cells should be rinsed in PBS. Incubate the fixed cells (on slides or in suspension) for 10-20 minutes in PBS containing 10-200 nM of probe. Note that the loading concentration is lower and staining time is shorter than in the procedure for staining live cells. After the incubation, wash the cells at least once with fresh PBS.

Fixation and Permeabilization after Staining with MitoTracker Red

After staining live cells with MitoTracker Red, it is often convenient to fix the cells in formaldehyde and to permeabilize them with Triton® X-100. For example, fixation and permeabilization makes it possible to probe for other intracellular structures by immunocytochemistry. Mitotracker®Red is well-retained following fixation and permeabilization using the protocol described below

2.1 Washing the cells. After staining, wash the cells in fresh, prewarmed growth medium. This step is especially important if the cells are attached to a BD Cell-Tak Adhesive–coated coverslip or another amine-containing surface.

2.2 Fixing the cells. Carefully remove the growth medium covering the cells, and replace it with freshly prepared, prewarmed growth medium containing 3.7% formaldehyde. Note, if the growth medium contains serum, then the formaldehyde solution should be prepared in growth medium containing serum. Incubate at 37°C for 15 minutes.

2.3 Rinsing the cells. After fixation, rinse the cells several times in PBS.

2.4 Permeabilization. When the cells are going to be subsequently labeled with an antibody, a permeabilization step is usually required to enhance the antigen's accessibility. Incubate the fixed cells in PBS containing 0.2% Triton X-100 at room temperature for 5 minutes. Following permeabilization, rinse the cells in PBS. Alternatively, the cells may be permeablilized by incubating in ice-cold acetone for 5 minutes, and then washed in PBS. Even when cells are not going to be labeled with an antibody, this acetone-permeabilization step may be useful because it appears to improve signal retention.

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References

1. J Histochem Cytochem 44, 1363 (1996);
2. Cytometry 39, 203 (2000);
3. J Biochem (Tokyo) 121, 29 (1997);
4. Anal Biochem 279, 142 (2000);
5. Mol Cell Biochem 172, 171 (1997).

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