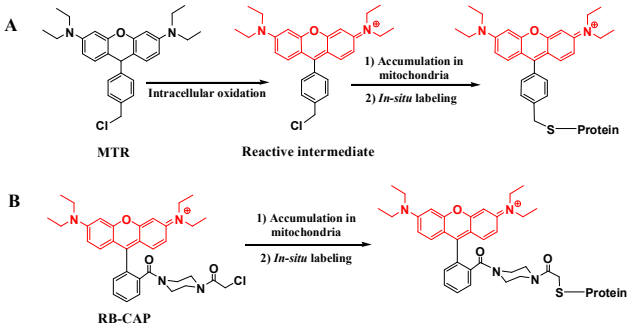
BIOLUMINOR

MitoTracker Red RB-CAP

The cell-permeant MitoTracker probe contains a thiol-reactive chloromethyl moiety for covalent mitochondrial labeling. RB-CAP is electrophoretically accumulated into mitochondria and formed covalent bioconjugates with intramitochondrial sulfhydryls. RB-CAP is highly photostable and is retained in the mitochondria after fixation.

Lipophilic and cationic dyes, e.g. rhodamine 123 and JC-1M, could be accumulated in mitochondria driven by the negative mitochondrial transmembrane potential. The aforementioned staining of mitochondria quickly disappears upon loss of the mitochondrial transmembrane potential.

MitoTracker probes with a chloromethyl moiety have been developed to overcome this limitation,. The reactive handle forms covalent bioconjugates with intramitochondrial protein sulfhydryls. The covalent linkages between MitoTrackers and proteins prevent the loss of intramitochondrial fluorescence at dissipation of the membrane potentials. RB-CAP (Scheme 1B). RB-CAP exhibits superior labeling characteristics than commercial MitoTracker Red (Scheme 1A0.



Scheme 1. Fluorescent labeling of intra-mitochondrial proteins with RB-CAP as compared to MitoTracker Red.

Photophysical properties of RB-CAP

Fig. 1 shows that the optimal fluorescence excitation of RB-CAP is at 560 nm while the maximal fluorescence emission intensity is at 580 nm. RB-CAP exhibits superior photostability over commercial MitoTrackers inside cells.

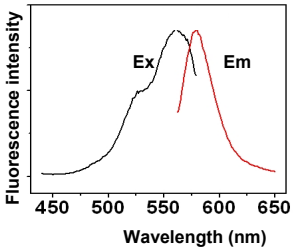


Fig. 1 Fluorescence excitation and emission spectra of RB-CAP in PBS buffer. The fluorescence emission spectrum was obtained using *λex*@560 nm, and the fluorescence excitation spectrum was scanned using *λem*@580 nm.

Materials Required but Not Provided

DMSO Suitable buffer or growth medium for live cell imaging
Fixatives such as formaldehyde for cell fixation
Detergents such as Triton® X-100

Preparing Stock Solutions

Before opening a vial, allow the product to warm to room temperature.

To prepare a stock solution, dissolve the product in high-quality, anhydrous dimethyl-sulfoxide (DMSO) to a final concentration of 1-10 mg ml^{-1}. It is preferable to use solutions immediately after they are prepared. Store the solutions of the dye frozen at -20 °C and protected from light.

Cell Preparation and Staining

The concentration of probe for optimal staining varies by applications. The initial condition suggested here is 1-10 µg ml^{-1}.

1.1 Preparing staining solutions: Dilute 1-10 mg ml^{-1} stock solution to the final working concentration in appropriate buffer or grow medium.

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 confluency, remove the media from dish and add pre-warmed (37°C) staining solution containing RB-CAP. While incubation times vary depending on the model system and probe used incubation for 15–45 min under growth conditions appropriate for the particular cell type is generally sufficient but may need to be optimized. After staining is complete replace the staining solution with fresh prewarmed media or buffer and observe cells using a fluorescence microscope or fluorescence microplate reader. If the cells are to be fixed and permeabilized, continue to Fixation and Permeabilization after Staining.

Staining suspension cells. Centrifuge to obtain a cell pellet and aspirate the supernatant. Resuspend the cells gently in pre-warmed (37°C) staining solution containing RB-CAP (prepared in step 1.1). While incubation times vary depending on the model system and probe used, incubation for 15–45 min under growth conditions appropriate for the particular cell type is generally sufficient but may need to be optimized. After staining is complete, re-pellet the cells by centrifugation and resuspend cells in fresh prewarmed medium or buffer. Cells maybe analyzed by flow cytometry, microplate-based analysis, or fluorescence microscopy. If immobilized cells on coverslips are needed, use poly-D-lysine to coat the slides or coverslips before mounting. If the cells are to be fixed and permeabil ized, continue to Fixation and Permeabilization after Staining.

Optional: Fixation and Permea-bilization after Staining.
After staining live cells with RB-CAP, it is often useful to fix and permeabilize the cells for subsequent manipulations. For example, fixation and permeabilization allows you to probe for other intracellular structures by immuno cytochemistry.

2.1 Washing the cells. After staining, wash the cells in fresh, pre-warmed buffer or growth medium.

2.2 Fixing the cells. Carefully remove the medium/buffer covering the cells, and replace it with freshly prepared, pre-warmed buffer or growth medium containing 2–4% formaldehyde.

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

2.4 Permeabilization (optional).
When permeabilization is needed for subsequent steps such as immunocytochemistry, incubate fixed cells in buffer containing detergent such as Triton® X-100. Following permeabilization, rinse the cells in buffer and proceed with immunocytochemistry procedure. We found that incubating the endothelial cells for 10 min in PBS containing 0.2% Triton® X-100 works well. Alternatively, the cells may be permeabilized by incubating in ice-cold acetone for 5 min, and then washed in PBS. Even when cells are not going to be labeled with an antibody, this acetone-permeabilization step may be useful in improving the signal-to-background ratio.

References

1. *J. Histochem. Cytochem.*, 1996, 44, 1363
2. *Anal. Methods*, 2012, 4, 1699

厦门火炬高新区（翔安）产业区台湾科技企业育成中心 E1003 室
Tel: (0592)7099338 Fax: (0592)7083338
Website: www.bioluminor.com
Email: sales@bioluminor.com