abberior LIVE ORANGE mito for imaging cristae structure

abberior offers a variety of excellent fluorescent dyes with optimized properties for the labeling of biomolecules, spectroscopic studies, optical microscopy, and particularly optical nanoscopy featuring STED super-resolution.

Our abberior LIVE ORANGE mito probe can be readily used to specifically label the cristae in mitochondria of living cells. This bright and photostable probe allows the observation of natural behavior of substructures inside mitochondria with super resolution STED microscopy. Minimal bleaching and reduced toxcity of this probe allows long term imaging of mitochondria in living speciments.

In combination with our abberior LIVE dyes, you get a toolbox of super-efficient probes, that provide specific labeling of intracellular targets and outer cell membrane, making long-term multi color live-cell imaging possible. Additionally extremely low nanomolar probe concentrations reducing potential toxic effects and cellular stress.

Storage

Our abberior LIVE ORANGE mito probe are freeze-dried and are shipped at room temperature. Upon arrival, the product can be stored for up to one year at -20 °C. Proximately before the staining procedure dissolve the probe in DMF or DMSO. Once dissolved, the stock solution should be kept at –20 °C, protected from light and moisture.

Note: Depending on solvent quality the shelf-life of the stock solution might be significantly reduced compared to the probe in its solid form – even if stored at -20 °C.

Staining of the cristae structure using abberior LIVE ORANGE mito probe

The procedure described below has been successfully tested with our abberior LIVE ORANGE mito probe in several adherent mammalian cultered cell lines. These procedures may not be optimum for a certain experimental conditions but have yielded consistent results in most instances.

Required reagents / equipment; not provided:

- dry DMF or dry DMSO
- glass coverslips, glass-bottom dish, or similar imaging chamber with a glass thickness of ~170 µm (No. 1.5 or No. 1.5H)

Note: We do not recommend using plastic coverslips or live-cell chambers with plastic bottoms because only suboptimal imaging results are achieved. If possible, coverslips with grids, gratings or similar should be avoided, as these structures can interfere with imaging causing aberrations that degrade image quality

- Live-cell imaging medium (e.g. evrogen DMEM^{gfp}-2)
- Tweezer
- Optional: Cavity slide or coverslip holder
- Optional: silicone glue (e. g. Twinsil, Picodent)
- Fluorescens microscope with live-cell incubator and suited excitation light and detection filter

Staining procedure for cultured cells

Depending on the doubling time of the cell line, the seeding time must be determined. Seed cells in a desired density in a cell culture chamber or onto coverslips before labeling. Use standard cell culture medium that is optimal for the cell line.

Note: Cells grown in very high densities, i.e., a confluent layer, may give rise to high labeling background.

Staining and imaging will take place on the same day

1. Prewarm the live-cell imaging media to the optimal temperature required to cultivate the desired cell line. In most cases this would be 37 °C.

2. Prepare a stock solution of 1 mM by desolving the probe in 30 µl of DMF or DMSO.

   Note: If you´re using an evaluation sample, dissolve the substance in 2 µl DMF or DMSO to receive a concentration of 1 mM.

3. Prepare the staining solution using prewarmed live-cell imaging medium. A final concentration of 250 to 500 nM is recommended. The required concentration for proper labeling strongly depends on the used cell type.

   Optional: Our extended LIVE probes can be combined with our abberior LIVE ORANGE mito probe. Simply add them to the staining solution for multocolor live-cell imaging.

   Note: The staining solution is not stable for extended periods of time. Therefore, it is recommended that you only prepare enough solution for immediate use.

4. Remove the cell culture medium and rinse the cells once in prewarmed live-cell imaging medium.

5. Remove the medium and add enough staining solution to the cells. Incubate for 45 to 60 min at optimal cell growth conditions (temperature, humidity, CO_2-controlled enviroment).

6. Afterwerds, cells are rinsed three times with fresh live-cell imaging medium. Followed by an additional 15 to 20 minutes washing step in fresh live cell imaging medium.

7. Cells are embedded in fresh live-cell imaging medium.

   If coverslips were used: simply take the coverslip out of the staining solution using tweezers. Place the coverslip in a

   i. coverslip holder or

   ii. mount it onto a cavity slide (cells facing downwards) which is filled with fresh live-cell imaging medium.

In case of (ii) remove the excess imaging medium using tissue paper. Gently press down the coverslip to prevent it from moving. The mounted sample can be sealed using silicone glue (e. g. Twinsil, *Picodent*).

8. After staining and embedding, the samples should be immediately imaged on a microscope equipped with a live-cell incubator.

   Note: For live-cell imaging cells must always be kept at ambient conditions (temperature, humidiy, pH and CO_2-conditions). This is particularly important for long-term measurements.

Abbreviations

DMF N,N-Dimethylformamid

DMSO Dimethylsulfoxide

STED Stimulated Emission Depletion