

Product information: Mito Flipper-TR[®] (SC023)

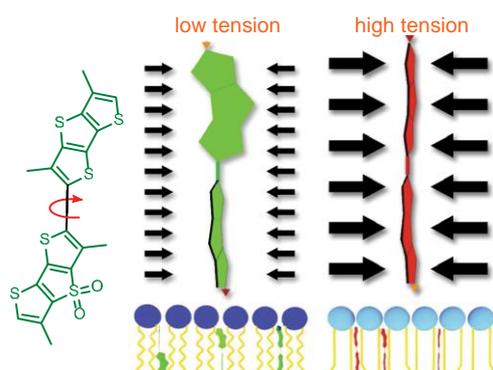
Live Cell Mitochondria Membrane Tension Probe

Introduction

Mito Flipper-TR[®] is a live cell fluorescent probe that specifically targets the Mitochondrial membrane of cells and reports membrane tension changes through its fluorescence lifetime changes. It is one of the targeted Flipper probes family¹⁻³⁾ which sense changes of the organization of lipid bilayer membranes through changes of the twist angle and polarization between the two twisted dithienothiophenes of the mechanophore. Mito Flipper-TR[®] spontaneously localizes to the mitochondrial membrane of cells and is only fluorescent when inserted in the lipid membrane. It has a broad absorption and emission spectrum, excitation can be commonly performed with a 488nm laser, while emission is collected between 575 and 625nm.

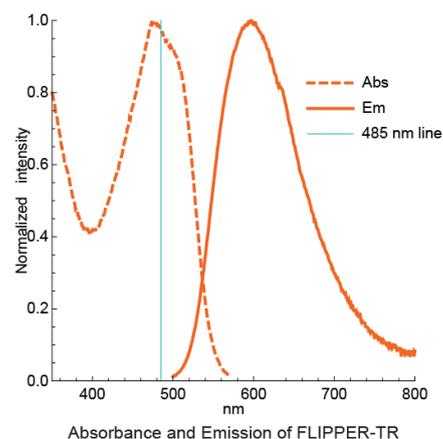
Photophysical properties

| | |
|------------------|---|
| λ_{Abs} | 480 nm |
| λ_{Em} | 600 nm |
| ϵ_{max} | $1.66 \cdot 10^4 \text{ mol}^{-1} \cdot \text{cm}^{-1}$ (DMSO) |
| lifetime | 2.8 - 7 ns |
| QY | 30% (AcOEt) |



Storage & Handling

Store the probe at -20°C upon receipt. Prepare solutions of the probe using new and anhydrous DMSO (as old and wet DMSO can strongly reduce the shelf life of the probe). Store solutions of the probe at -20°C after use. Vials should be allowed to warm to room temperature before opening. When stored properly, the probe in solution is stable for up to 3 months. Note: DMSO solutions should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. Dispose of these reagents in compliance with all pertaining local regulations.



Labelling Protocol

Note: This protocol was optimized using HeLa cells adhering to coverslips and has been confirmed in other common cell lines. Recommendations for experimental protocols should be used as a starting point, and optimal labeling conditions for each cell type should be determined empirically.

Prepare 1 mM stock solution. Dissolve the content of the vial of Mito Flipper-TR[®] in 35 μL of anhydrous DMSO to make a 1 mM stock solution. This solution should be stored at -20°C or below. Do not divide the solution into small aliquots, they will decay faster and the compound is not altered by multiple freeze-thaw cycles. When stored properly, this stock solution is stable for up to three months. (Optional) If the concentration of the stock solution needs to be accurately determined, dilute 1 μL of 1 mM stock solution in 99 μL of DMSO. Measure the absorbance at 425 nm. Calculate the concentration using the extinction coefficient given above.

Prepare staining solution. Dilute Mito Flipper-TR[®] DMSO stock solution to the desired concentration (start with 1 μM) in cell culture medium shortly before applying to the cells (Apply quickly, max 5 minutes, the staining solution to the cells of which the growth medium was removed).

Note: when using a cell culture media supplemented with Fetal Calf Serum (FCS) or other serum proteins, the efficiency of labelling will be reduced compared to media devoid of serum. If a low signal is observed, the probe concentration can be increased up to 2-3 μM .

Cell preparation and staining. Grow cells on coverslips, glass bottom dish or glass bottom multi-well plates as usual. When cells have reached the desired density, replace the culture medium by the **staining solution** (prepared shortly before) ensuring that all the cells are covered with solution. Place the cells in the incubator at 37°C in a humidified atmosphere containing 5% CO₂

for 15 minutes before imaging. Optionally, the medium containing the probe can be removed, and cells washed once in fresh growth media. As the probe is fluorescent only in membranes, the probe does not need to be removed, especially in cases where the staining medium contains serum when long term imaging (>24h) is planned. No impact on cell viability has been observed on HeLa cells at concentrations up to 5 μ M.

FLIM imaging. Cells are imaged with standard FLIM microscopes using a 485 or 488 nm pulsed laser for excitation and collecting photons through a 600/50 nm bandpass filter. We recommend optimizing the labeling procedure as well as the image acquisition settings to minimize photodamage induced by the 488nm excitation light on live samples. To extract lifetime information, the photon histograms from ROI or single pixels (accumulate sufficient counts to ensure good statistics) are fitted with a double-exponential, and two decay times, τ_1 and τ_2 are extracted. The longest lifetime with the higher fit amplitude τ_1 is used to report membrane tension and varies between 2.8 and 7.0 ns. Longer lifetime means more tension in the membrane. τ_2 with a smaller value (between 0.5 and 2 ns) and a small fit amplitude is less suited to study membrane tension. The lifetime can be correlated to absolute membrane tension using the calibration procedure given in Reference 1. In HeLa cells, average τ_1 lifetimes in mitochondrial membranes were around 3.2 ns while 3.3 ns in COS7 cells. Hyperosmotic shock (0.5 M sucrose) lowers lifetimes by ~0.2 ns.³⁾

Important notes:

- Membrane tension measurements can only be performed by FLIM microscopy, fluorescence intensity or wavelength is not reliably reporting on membrane tension.
- Systems where the membrane lipid composition changes over time may also induce a change of Mito Flipper-TR[®] lifetime.
- FLIM imaging is an advanced microscopy technique requiring a commercial or custom built FLIM microscopy system with the adequate excitation lasers, photon counting systems and emission filters. Customers are advised to consult their instrument responsible person or contact the microscope manufacturer to ensure that their system is able to image Mito Flipper-TR[®] fluorescence and lifetime.

References:

- 1) Colom A, *et al*: A fluorescent membrane tension probe. *Nat Chem*, 2018, **10**:1118–1125 ().
- 2) Dal Molin M, *et al*: Fluorescent flippers for mechanosensitive membrane probes. *JACS*, 2015, **137**:568-571.
- 3) 2) Goujon A, *et al*: Mechanosensitive Fluorescent Probes to Image Membrane Tension in Mitochondria, Endoplasmic Reticulum, and Lysosomes, *J. Am. Chem. Soc.* 141, 8, 3380–3384, 2019.

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