

Product information: ER Flipper-TR[®] (SC021)

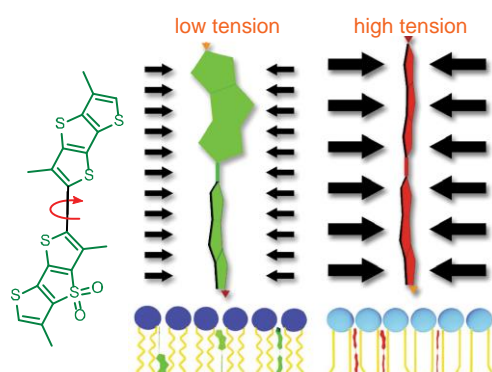
Live Cell Endoplasmic Reticulum Membrane Tension Probe

Introduction

ER Flipper-TR[®] is a live cell fluorescent probe that specifically targets the endoplasmic reticulum (ER) 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. ER Flipper-TR[®] spontaneously localizes to the ER 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.

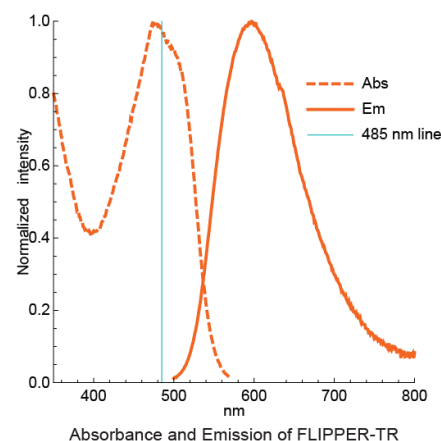
Photophysical properties

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 labelling conditions for each cell type should be determined empirically.

Prepare 1 mM stock solution. Dissolve the content of the vial of ER 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 ER 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% CO_2

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 lifetimes in ER membranes were around 3.4 ns but could vary and show 4.1 ns (tubules) down to 3.8-3.2 ns (sheets), depending also on cells and conditions. Hyperosmotic shocks (0.5 M sucrose) lower lifetimes by 0.2-0.5 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 ER 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 ER 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.

ER Flipper-TR[®] probe is distributed by Spirochrome under an exclusive license from the University of Geneva, Switzerland and was developed by the NCCR Chemical Biology.

Spirochrome products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply. Spirochrome products and product applications are covered by patents and patents pending.

Limited Use Label License: For research use only. Not intended for any animal or human therapeutic or diagnostic use. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Spirochrome will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Spirochrome is willing to accept return of the unused product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Spirochrome: Spirochrome AG, Postfach 213, 8620 Stein am Rhein, Switzerland, Email: info@spirochrome.com