

## Product information: PKmito ORANGE FX (SC054)

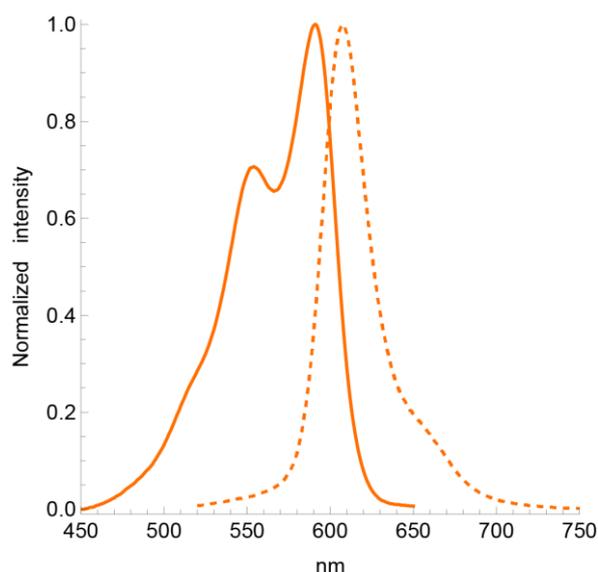
Fixed Cells Mitochondrial Probe With High STED compatibility

### Introduction

PKmito Orange FX (also known as PKMO FX) is a bright, photostable, mitochondrial probe based on the PKmito™ dyes developed in the lab of Zhixing Chen at Peking University<sup>1</sup>. PKmito Orange FX labels mitochondria in formaldehyde (FA) or glutaraldehyde (GA) fixed cells with very high specificity. The unique and unmatched feature of PKmito Orange FX is its ability to be retained nearly quantitatively after aldehyde fixation of stained cells. PKmito Orange FX is extremely well suited for STED and SIM superresolution imaging. It allows to perform nanoscopy of mitochondria with exquisite resolution and bright signal. PKmito Orange FX accumulates in the mitochondrial inner membrane (IM) and stays in place upon fixation. It is highly suited to image mitochondrial cristae structure by STED superresolution microscopy using a 775 nm depletion line. PKmito Orange FX does not require any genetic manipulation, transfection or overexpression of fluorescent proteins. PKmito Orange FX enables multicolor imaging with SPY505, SPY650, SPY700, SiR or GFP. It can be used for widefield, confocal, SIM or STED imaging in fixed cells and tissue. Contains 1 vial of PKmito Orange FX (lyophilized).

### Probe Properties

|   |                  |
|---|------------------|
| <b>Absorbance maximum <math>\lambda_{\text{abs}}</math> (MeOH)</b>  | 584 nm           |
| <b>Fluorescence maximum <math>\lambda_{\text{fl}}</math> (MeOH)</b> | 604 nm           |
| <b>Works on fixed cells?</b>  | Yes              |
| <b>Probe quantity</b>   | 100 stainings*   |
| <b>Fluorescence lifetime (in cells)</b>                             | n.d.             |
| <b>STED depletion wavelength</b>                                    | 775 nm           |
| <b>Shipping</b>   | room temperature |
| <b>Storage</b>  | -20°C            |



### Storage & Handling

Store the probe at -20°C or below upon receipt. The lyophilized probe is stable for >1 week at room temperature and for >12 months at -20°C. Reconstitute PKmito ORANGE FX using anhydrous DMSO. We recommend using newly or freshly opened and anhydrous DMSO to prepare the 1000x stock solution. In contact with air and moisture, DMSO produces decay products which can strongly reduce the shelf life of the probe in solution, even at -20°C. Keep the 1000x stock solution of the probe at -20°C after use. Vials should be allowed to warm to room temperature before opening. When reconstituted and stored properly, the 1000x stock solution is stable for 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 in this protocol should be used as a starting point, and optimal labeling conditions for each cell type should be determined empirically. The recommended staining dilution is 1000 fold and it can be adapted depending on the specific needs of the cell line or imaging conditions.

**1. Prepare 1000x stock solution.** Add 50  $\mu\text{L}$  of anhydrous DMSO to the PKmito ORANGE FX vial to prepare the 1000x stock solution (the stock solution has an absolute concentration of 500  $\mu\text{M}$ ). We recommend using newly or freshly opened and anhydrous DMSO to prepare the 1000x stock solution. In contact with air and moisture, DMSO produces decay products which can

strongly reduce the shelf life of the probe in solution, even at -20°C. After use, this solution should be stored at -20°C or below. Do not divide the 1000x stock solution into small aliquots. The probe is not altered by many freeze-thaw cycles. When stored properly, this stock solution is stable for 3 months.

**2. Prepare the staining solution.** Dilute PKmito ORANGE FX to 1x (= 500 nM) in your usual cell culture medium (e.g. DMEM + 10% fetal bovine serum) and vortex briefly. Proceed quickly to step 3. Since staining efficiency can depend on the cell line, it is recommended to stain cells with 1000x dilution at the first attempt and then optimize the PKmito ORANGE FX dilution factor in further experiments until an optimal staining is achieved. Use only freshly made staining solution, and do not use it multiple times.

**Note:** The staining efficiency may vary depending on the cell line used. In some cases it is necessary to add 5-10 uM verapamil (not provided) to the staining solution or increase labeling time to improve the staining.

**3. 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** freshly prepared under step 2 ensuring that all the cells are covered with the solution. Place the cells in the incubator at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> for 1-2h. Wash the cells 3x with PBS and perform fixation with preheated 2% - 2.5% glutaraldehyde (GA) or 4% paraformaldehyde (PFA) for a minimum of 10 min. Cells are then washed 3x with PBS. Optional: prior to fixation, the live cells can be quickly imaged to control if the staining was successful.

If immunostaining is planned, use the following protocol: after incubation with the staining solution for 2h, wash the cells with pre-warmed (37 °C) DMEM three times. Prefix the cells by immersion with pre-warmed 2% GA in 0.1 M phosphate buffer (pH 7.4) for 20 sec. Replace the pre-fixative solution with pre-warmed 4% FA in 0.1 M phosphate buffer (pH 7.4) and fix the cells for 8 min at RT. Exchange the fixative solution with 0.1 M phosphate buffer and keep the cells at RT for 10 min. Incubate the cells 0.1 M NH<sub>4</sub>Cl in 0.1 M phosphate buffer for 5 min. Permeabilize the cells with 0.25% Triton X-100 in 0.1 M for 5 min and wash 5 times with PBS. Immunolabeling can then be performed using standard protocols.

**4. Cell imaging.** After cell staining following the instructions under **3.**, Imaging of PKmito ORANGE FX is best performed using 580-590 nm excitation and reading fluorescence between 600 and 700 nm but the settings can be optimized depending on the experiment (e.g. multicolor imaging). PKmito ORANGE FX can be imaged by STED nanoscopy, using a 775nm depletion laser.

\* Based on the following conditions: 0.5 ml staining solution / staining experiment with 1x probe concentration. The number of staining experiments can be further increased by reducing volume or probe concentration.

1. Jingting Chen et Al. "An Aldehyde-crosslinking Mitochondrial Probe for Nanoscopic Imaging in Fixed Cells", PNAS (2024)

PKmito™ 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. PKmito™ products are Nanjing Genvivo Biotech Co. Ltd. products sold by Spirochrome under an exclusive license. PKmito is a registered trademark of Nanjing Genvivo Biotech Co. Ltd.

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, Chalberweidstrasse 4, 8620 Stein am Rhein, Switzerland, Email: [info@spirochrome.com](mailto:info@spirochrome.com)