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Product information: PKmito ORANGE FX (SC054)

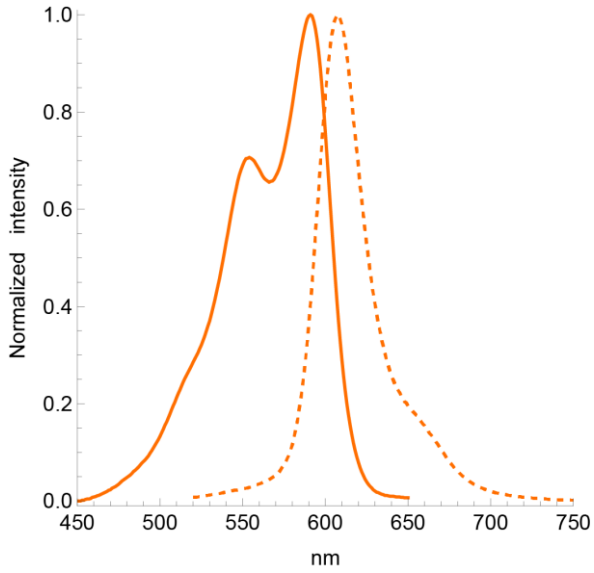
Fixed Cells Mitochondrial Probe With High STED compatibility

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[1)]. 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 mitochonridal 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

Absorbance maximum λ_{abs} (MeOH)	584 nm
Fluorescence maximum λ_{fl} (MeOH)	604 nm
Works on fixed cells?	Yes, PFA and GA
Probe quantity	100 stainings*
Fluorescence lifetime (in cells)	n.d.
STED depletion wavelength	775 nm
Shipping	room temperature
Storage	-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 µL of anhydrous DMSO to the PKmito ORANGE FX vial to prepare the 1000x stock solution (the stock solution has an absolute concentration of 500 uM). 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

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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_2 for 1-2h. Wash the cells 3x with PBS and perform fixation with preheated 2% - 2.5% glutaraldehyde (GA) or 4% paraformaldehyde (PFA) for at 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_4Cl 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)