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Product information: PKmito RED (SC052)

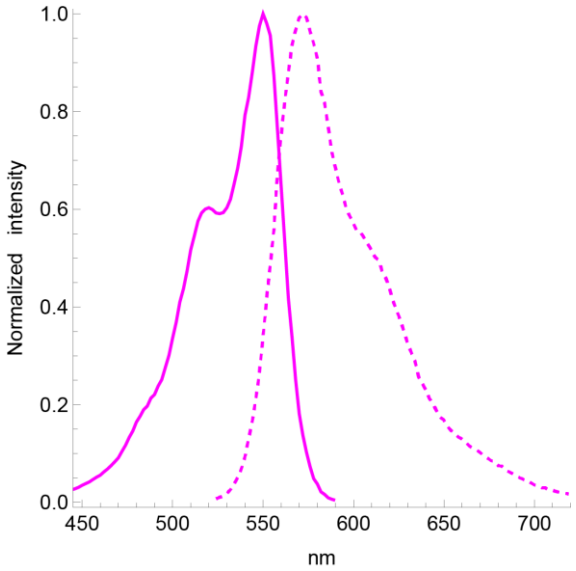
Live Cell Mitochondrial Probe With Very Low Phototoxicity

Introduction

PKmito RED is a bright, non-phototoxic & non-toxic mitochondrial probe based on the PKmito™ dyes developed by by Yang et al in the lab of Zhixing Chen[1)]. PKmito RED labels mitochondria in live cells with very high specificity. The unique and unmatched feature of PKmito RED is its extremely low phototoxicity, due to the presence of the intramolecular triplet quencher cyclooctatetraene (COT) group. It allows to perform long term imaging of mitochondria without damaging them. PKmito RED accumulates in the mitochondrial inner membrane (IM) and can used to image mitochonridal cristae by STED or SIM superresolution microscopy. PKmito RED does not require any genetic manipulation, transfection or overexpression of fluorescent proteins. PKmito RED enables multicolor imaging with SPY505, SPY595, SPY650, SPY700, SiR or GFP. PKmito RED can be imaged with a standard TMR or Cy3 filterset. It can be used for widefield, confocal, SIM or STED imaging in living cells and tissue. Contains 1 vial of PKmito RED (lyophilized).

Probe Properties

Absorbance maximum λ_{abs} (MeOH)	549 nm
Fluorescence maximum λ_{fl} (MeOH)	569 nm
Works on fixed cells?	No
Probe quantity	100 stainings*
Fluorescence lifetime (in cells)	0.8 ns
STED depletion wavelength	660 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 RED 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. PKmito RED accumulates in mitochondria of live cells. It may therefore modify mitochondria metabolism in living cells if it is used above 1:400 dilution. The recommended staining dilution is 1000 fold or higher if long term (>12h) imaging experiments are planned. For all other purposes, 1000 fold dilution PKmito RED for staining is recommended. For labelling tissue samples a 1:500 dilution is recommended.

1. Prepare 1000x stock solution. Add 50 µL of anhydrous DMSO to the PKmito RED vial to prepare the 1000x stock solution. 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, they will decay faster and 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 RED to 1x 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 RED dilution factor in further experiments until an optimal staining is achieved. Use only freshly made staining solution, and do not use it multiple times.

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 30-60 minutes (minimum 15 minutes). Then exchange the staining solution covering the cells with fresh cell culture medium (= without PKmito RED) once.

4. Cell imaging. After cell staining following the instructions under **3.**, Imaging of PKmito RED is best performed using standard Cy3 settings. Additional washing steps are optional if some strong fluorescence background is observed.

* 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.

** These labelling times were determined for HeLa cells and may differ depending on the cell line used.

1. Yang, Zhongtian, et al. "Cyclooctatetraene-conjugated cyanine mitochondrial probes minimize phototoxicity in fluorescence and nanoscopic imaging." Chemical science 11.32 (2020): 8506-8516.

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