

## Product information: PKmito DEEP RED (SC055)

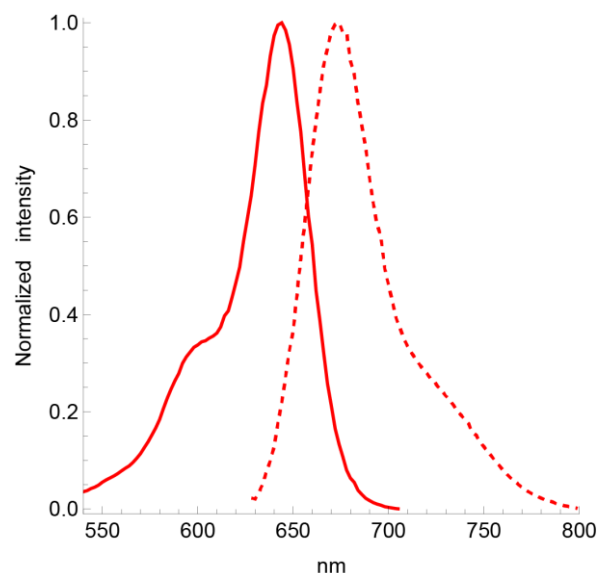
Live Cell Mitochondrial Probe With Very Low Phototoxicity

### Introduction

PKmito DEEP RED is a bright, non-phototoxic & non-toxic mitochondrial probe based on the PKmito™ dyes developed by Yang et al in the lab of Zhixing Chen<sup>1</sup>. PKmito DEEP RED labels mitochondria in live cells with very high specificity. The unique and unmatched feature of PKmito DEEP 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 DEEP RED accumulates in the mitochondrial inner membrane (IM) and can be used to image mitochondrial cristae by STED or SIM superresolution microscopy. PKmito DEEP RED does not require any genetic manipulation, transfection or overexpression of fluorescent proteins. PKmito DEEP RED enables multicolor imaging with SPY505, SPY555, SPY595, SPY620 or GFP. PKmito DEEP RED can be imaged with a standard Cy5 filterset. It can be used for widefield, confocal, SIM or STED imaging in living cells and tissue. Contains 1 vial of PKmito DEEP RED (lyophilized).

### Probe Properties

<b>Absorbance maximum <math>\lambda_{abs}</math> (MeOH)</b>	644 nm
<b>Fluorescence maximum <math>\lambda_{fl}</math> (MeOH)</b>	670 nm
<b>Works on fixed cells?</b>	No
<b>Probe quantity</b>	100 stainings*
<b>Fluorescence lifetime (in cells)</b>	0.7 ns
<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 DEEP 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 DEEP 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 DEEP 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 DEEP 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 DEEP 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 DEEP 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<sub>2</sub> for 30-60 minutes (minimum 15 minutes). Then exchange the staining solution covering the cells with fresh cell culture medium (= without PKmito DEEP RED) once.

**4. Cell imaging.** After cell staining following the instructions under **3.**, Imaging of PKmito DEEP RED is best performed using standard Cy5 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.

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 and product applications are covered by patents and patents pending. 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, Postfach 213, 8620 Stein am Rhein, Switzerland, Email: [info@spirochrome.com](mailto:info@spirochrome.com)