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Product information: PKmem 590 (SC063)

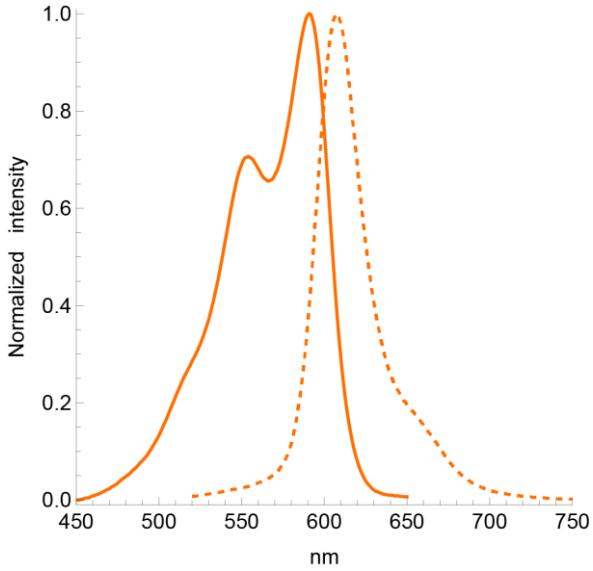
Live & fixed Cell Plasma Membrane Probe With Very Low Phototoxicity and high STED compatibility

Introduction

PKmem 590 is a bright, non-phototoxic & non-toxic plasma membrane probe based on the PKmem™ dyes developed by the lab of Zhixing Chen at Peking University[1]. PKmem 590 labels the plasma membrane in live cells with very high specificity. The unique and unmatched feature of PKmem 590 is its extremely low phototoxicity, due to the presence of the intramolecular triplet quencher cyclooctatetraene (COT) group. It allows to perform long term imaging the plasma membrane without damaging the cells. It is highly suited to image plasma membrane structure and dynamics by STED superresolution microscopy using a 775 nm depletion line. PKmem 590 does not require any genetic manipulation, transfection or overexpression of fluorescent proteins. PKmem 590 enables multicolor imaging with SPY505, SPY555, SPY620, SPY650, SPY700, SiR or GFP. It can be used for widefield, confocal, SIM or STED imaging in living cells and tissue. Contains 1 vial of PKmem 590 (lyophilized).

Probe Properties

Absorbance maximum λ_{abs} (MeOH)	590 nm
Fluorescence maximum λ_{fl} (MeOH)	606 nm
Works on fixed cells?	No
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 PKmem 590 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 or KB cells adhering to coverslips and has been confirmed in other common cell lines (e.g. primary neuron, cardiomyocyte). Recommendations in this protocol should be used as a starting point, and optimal labeling conditions for each cell type should be determined empirically. PKmem 590 inserts itself into the plasma membrane of live cells. It may therefore modify plasma membrane metabolism in living cells if it is used above 1:200 dilution. The recommended staining dilution is 1000 fold or higher. For labelling tissue samples a 1:500 dilution is recommended.

1. Prepare 1000x stock solution. Add 50 µL of anhydrous DMSO to the PKmem 590 vial to prepare the 1000x stock solution (the stock solution has an absolute concentration of 100 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 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

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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 PKmem 590 to 1x in a serum free buffer or cell culture medium (e.g. Opti-MEM, PBS, HBSS, Tyrode's Solution or DMEM without fetal bovine serum. Neurobasal medium is also suitable) and vortex briefly. Proceed as quickly as possible to step 3 to obtain the best results. 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 PKmem 590 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.

3.1 Live cell staining: Grow cells on coverslips, glass bottom dish or glass bottom multi-well plates as usual. When cells have reached the desired density, Remove the culture medium of the cells, wash the cells with HBSS once and cover the cells with the freshly diluted PKmem™ probe solution from step 2. Place the cells in the incubator at 37°C in a humidified atmosphere containing 5% CO_2 for 5-15 minutes. Optional: exchange the staining solution covering the cells with HBSS or fresh cell culture medium (= without PKmem 590) once.

3.2 Fixed cell staining: Grow cells on coverslips, glass bottom dish or glass bottom multi-well plates as usual. When cells have reached the desired density, the cells can either be stained first with PKmem 590 as in step 3.1 and then fixed with 4% formaldehyde or 2% glutaraldehyde using standard protocols. Post fixation staining of cells fixed first with 4% formaldehyde or 2% glutaraldehyde (standard protocols), washed 3x with PBS and then stained with PBS containing 1x PKmem 590 for 15 minutes and washed with PBS.

4. Cell imaging. After cell staining following the instructions under **3.**, Imaging of PKmem 590 is best performed using 580-590 nm excitation and reading fluorescence between 600 and 700 nm but the setting can be optimized depending on the experiment (e.g. multicolor imaging).

* 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. Jing Ling et al. “A gentle palette of plasma membrane dyes”, BioRxiv, 2024