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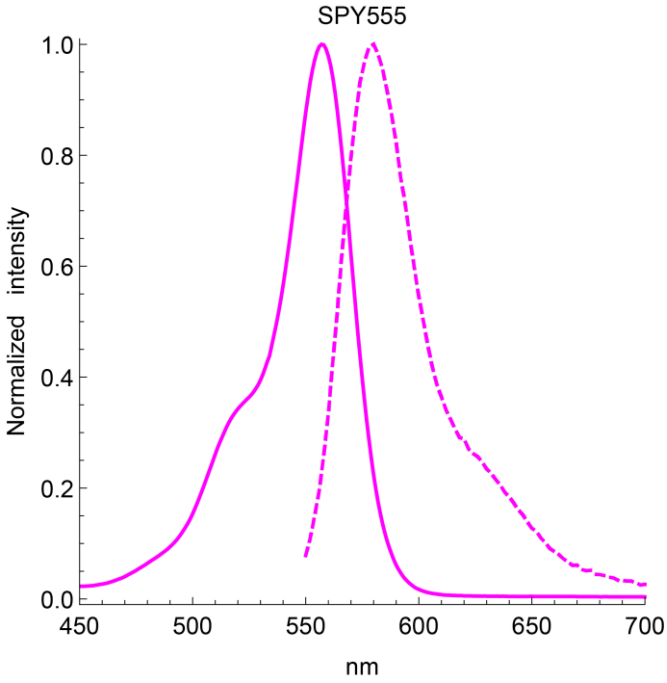
Product information: Peroxi_SPY555 (SC207)

Live Cell Probe for Peroxisomes

Introduction

Peroxi_SPY555 is a bright, orange & non toxic live cell peroxisome probe based on our SPY™ dyes series. Its optimized structure allows quick labeling of peroxisomes in live cells and tissues with high specificity and low background. Peroxi_SPY555 stains peroxisomes in live cells without the need for genetic manipulation or overexpression of fluorescent proteins. Its absorbance and emission spectra are similar to TMR or Cy3. Peroxi_SPY555 enables multicolor imaging with SPY505, SPY595, SPY650, SPY700, SiR or GFP. Peroxi_SPY555 can be imaged with standard TMR or Cy3 filterset. It can be used for confocal, SIM or STED imaging of living cells and tissue. Contains 1 vial of Peroxi_SPY555 (lyophilized).



Probe Properties

Absorbance maximum λ_{abs}	555 nm
Fluorescence maximum λ_{fl}	580 nm
Works on fixed cells?	Yes, 4% PFA
Probe quantity	100 stainings*
Fluorescence lifetime	2.4 ns
STED depletion wavelength	775 nm
Shipping	room temperature
Storage	-20°C

Storage & Handling

Store the kit 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 Peroxi_SPY555 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 below -20°C after use. Vials should be allowed to warm to room temperature before opening. When reconstituted and stored properly, the 1000x stock solution should 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

Important Notes:

a) This protocol was optimized and validated using HeLa, U2OS, or HEK293T cells adhering to coated glass or polymer dishes. For other 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.

b) Peroxi_SPY555 is only mildly fluorogenic and should be imaged by microscopes that are capable of out of focus light exclusion, such as confocal, spinning disk, STED or light sheet microscopes. Peroxi_SPY555 will yield poor signal to noise ratio using widefield microscopy.

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1. Prepare 1000x stock solution. Add 50 µL of anhydrous DMSO to the Peroxi_SPY555 vial to prepare the 1000x stock solution (DMSO stock concentration is 1 mM). 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. At this stage, the solution can be colored or not, this has no influence on the performance of the probe. 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 multiple freeze-thaw cycles. When stored properly, this stock solution is stable for 3 months.

2. Prepare the staining solution. Dilute Peroxi_SPY555 to 1x (i.e. a final concentration 1 uM) in your usual cell culture medium (e.g. DMEM + 10% fetal bovine serum) and vortex briefly. If the dilution is not performed in a single step, please use DMSO to prepare the intermediate dilution as using aqueous buffers to prepare the intermediate dilution will lead to the formation of probe aggregates. 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 Peroxi_SPY555 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 15 minutes**. Optional fixation: cells can be washed twice with PBS, fixed with 4% PFA for 20 min at R.T. and washed with PBS twice. Permeabilization with 0.5% TritonX and standard IF can be performed.

4. Cell imaging. Imaging of Peroxi_SPY555 is best performed using standard Cy3 settings. After labelling, the live cells can be immediately imaged without the need for washing steps. Optionally, a simple washing step consisting of replacing once the labelling solution by fresh culture medium which does not contain the probe may improve the signal to noise ratio but the signal will gradually fade within 30 minutes. **If time lapse imaging is performed, it is recommended to keep the probe in the imaging medium during the whole experiment to get a constant signal. 1-2 hours after addition of the probe to the cells, peroxisome metabolization of the probe will lead to increased intracellular background signal and a reduced signal to noise ratio. Therefore, the probe should not be used for timelapse experiments exceeding 2h**.

* 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.

** The recommended labelling time weas determined for 2D cultured cells and may differ slightly depending on the cell line used or the sample type (e.g. spheroids, organoids or tissue).