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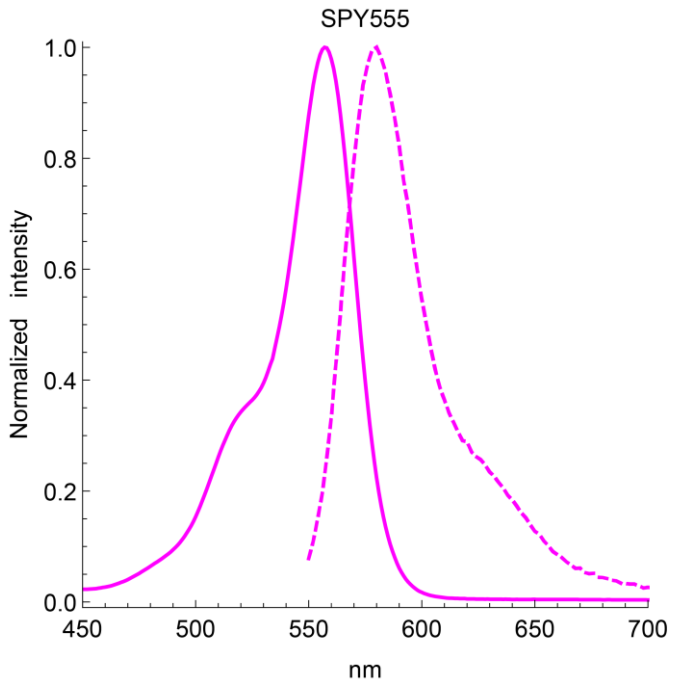
Product information: SPY555-CA (SC206)

SPY555-ChloroAlkane ligand for Halotag™* labeling

Introduction

SPY555-CA (also known as Map555-Halo) is a ChloroAlkane (CA) derivative of our SPY555 fluorophore. It emits light in the orange range, is fluorogenic and well suited for STED and SIM superresolution imaging. SPY555-CA can be imaged with a standard Cy3 settings. It can be used for widefield, confocal, SIM or STED imaging in living or fixed cells and tissue.
ChloroAlkane (CA) is the substrate of the self-labeling tag Halotag™*. Upon reaction with a CA derivative, Halotag™* forms a covalent bond with the substrate. It allows to permanently attach a fluorescent label to any protein of interest (POI) expressed as Halotag™* fusion.
Contains 1 vial of SPY555-CA (10 nmol, lyophilized).



Properties

Absorbance maximum λ_{abs}	555 nm
Fluorescence maximum λ_{fl}	580 nm
Works on fixed cells?	yes
Quantity	10 nmol
Fluorescence lifetime (HT7 bound)	2.3 ns
STED depletion wavelength	660 or 775 nm
Shipping	room temperature
Storage	-20°C

Storage & Handling

Store the SPY555-CA at -20°C or below upon receipt. The lyophilized Halotag™* substrate is stable for >1 week at room temperature and for at least 6 months at -20°C. Reconstitute SPY555-CA using anhydrous DMSO. We recommend using newly or freshly opened and anhydrous DMSO to prepare the stock solution. In contact with air and moisture, DMSO produces decay products which can strongly reduce the shelf life of the compound in solution, even at -20°C. Keep the stock solution of the SPY555-CA below -20°C after use. Vials should be allowed to warm to room temperature before opening. When reconstituted and stored properly, the 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: Recommendations in this protocol should be used as a starting point, and optimal labeling conditions for each cell type should be determined empirically.

1. Prepare DMSO stock solution. Add 50 µL of anhydrous DMSO to the SPY555-CA vial to prepare a 1000x (200 µM) stock solution. We recommend using newly or freshly opened and anhydrous DMSO to prepare the DMSO stock solution. In contact with

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air and moisture, DMSO produces decay products which can strongly reduce the shelf life of the substrate in solution, even at -20°C. At this stage, the solution can be colored or not, this has no influence on the performance of SPY555-CA. After use, this solution should be stored at -20°C or below. Do not divide the DMSO stock solution into small aliquots, they will decay faster and the compound 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 the SPY555-CA DMSO stock solution 1:1000 (final concentration 200 nM) in your usual cell culture medium (e.g. DMEM + 10% fetal bovine serum) and vortex briefly. Proceed quickly to step 3.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 may lead to the formation of aggregates. Since staining efficiency can depend on the cell line, it is recommended to stain cells with 1:1000 dilution at the first attempt and then optimize the dilution factor in further experiments until an optimal staining is achieved. The usual concentration range for live cell labelling is 100-500 nM. Use only freshly made staining solution and do not use it multiple times.

3. Cell preparation and staining. Grow cells transiently transfected or stably expressing a Halotag™* fusion-protein on coverslips, glass bottom dish or glass bottom multi-well plates as usual. When cells have reached the desired density or expression level of the Halotag™* fusion-protein, 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 min 30 minutes, although 1h is recommended.

Note: Before imaging, SPY555-CA stained cells can be fixed by any fixation method after the labelling step is completed. Additional immunolabeling or probe labeling can be performed after the fixation step using standard protocols.

4. Cell imaging. Imaging of SPY555-CA labeled cells 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 substrate may improve the signal to noise ratio. If the live cells were washed before imaging, the staining will last depending on your Halotag™* fusion protein stability and turnover rate.

*Halotag™ is a registered trademark of Promega

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