

Product information: CA-TestKit (SC300)

Fluorescent ChloroAlkane ligands for Halotag™* labeling

Introduction

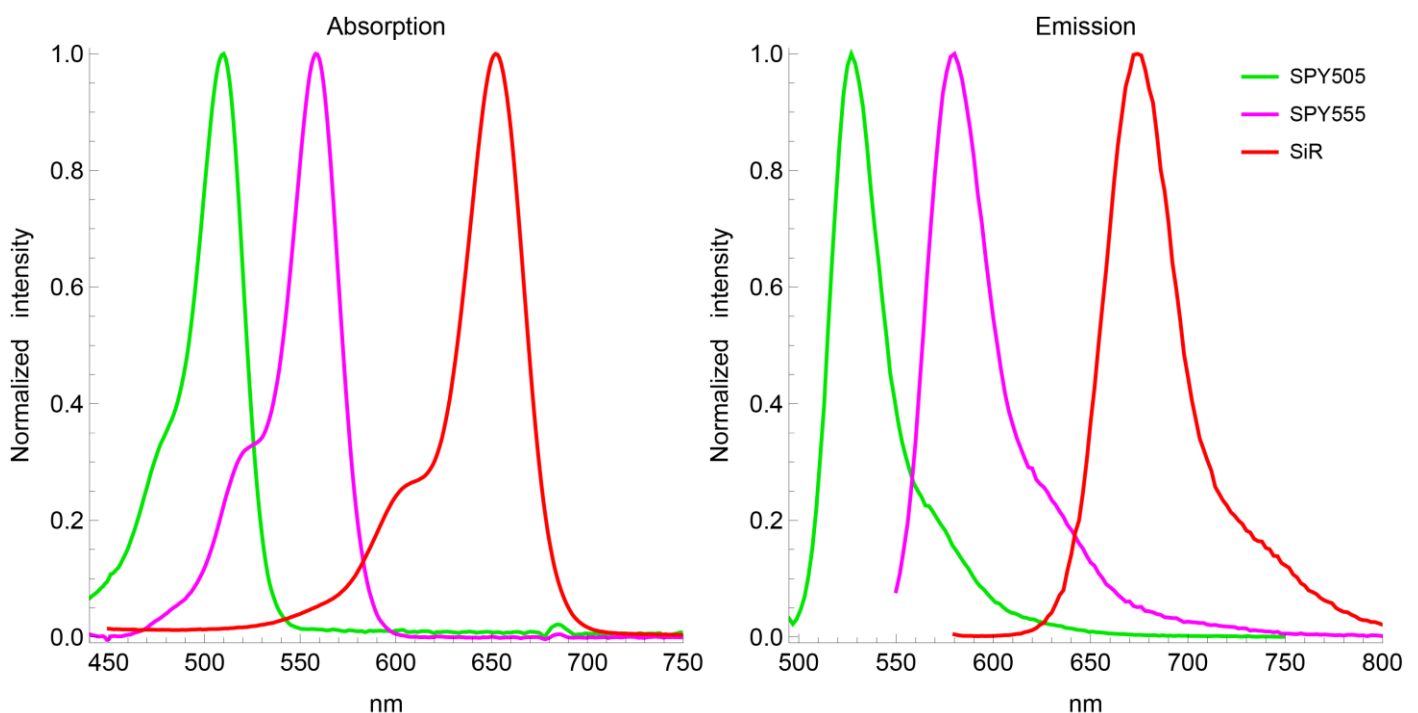
CA_TestKit combines our 3 Halotag™ substrates SPY505-CA, SPY555-CA and SiR-CA, offering a wide flexibility for Halotag™ fusion proteins labeling in live and fixed cells. The ligands are all bright, highly fluorogenic, and cell permeable. The 3 fluorophores are covering 3 distinct and very common imaging channels: FITC (green), TMR/Cy3 (orange) and Cy5 (far red).

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.

Kit contains 3 separate tubes containing 3 nmol of each Halotag™ substrate (ca. 30 stainings/tube**)

Properties

	SPY505-CA	SPY555-CA	SiR-CA
Absorbance maximum λ_{abs}	512 nm	555 nm	652 nm
Fluorescence maximum λ_{fl}	531 nm	580 nm	674 nm
Imaging "channel"	FITC or GFP	TMR or Cy3	Cy5
Works on fixed cells?	yes		
Quantity	3 nmol	3 nmol	3 nmol
Fluorescence lifetime	4.0 ns	2.3 ns	3.2 ns
STED depletion wavelength	660	660 or 775 nm	775 nm
Shipping	room temperature		
Storage	-20°C		



Storage & Handling

Store the CA_TestKit at -20°C or below upon receipt. The lyophilized Halotag™* substrates are stable for >1 week at room temperature and for at least 6 months at -20°C. Reconstitute the Halotag™* substrates 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 solutions of the Halotag™* substrates 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 15 µL of anhydrous DMSO to the Halotag™* substrate 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 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 the Halotag™* substrates. After use, the DMSO stock 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 Halotag™* substrate 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% CO₂ for min 30 minutes, although 1h is recommended.

Note: Before imaging, cells stained with the Halotag™* substrates 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 Halotag™* substrates labeled cells is best performed using settings that match the Abs/Em or “imaging channel” of each fluorophore given in the table above. 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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