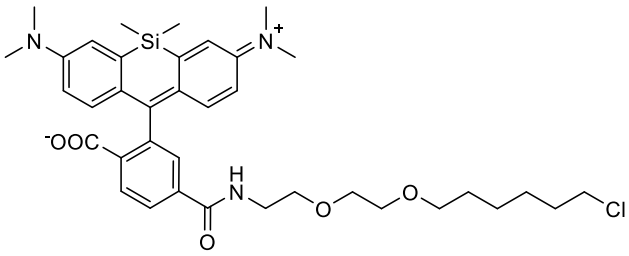
Spirochrome AG – Chalberwidstrasse 4
8260 Stein am Rhein - Switzerland
Email: info@spirochrome.com
Website: www.spirochrome.com

Product information: SiR-CA (SC506)

SiR-chloroalkane ligand for Halotag™* labeling

Introduction

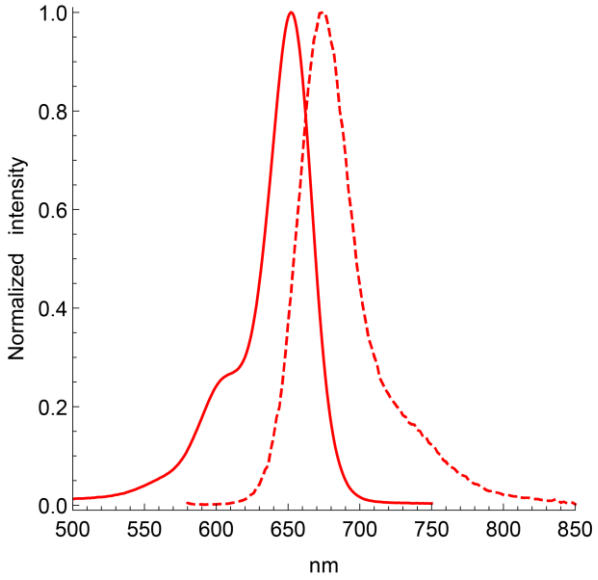
SiR-CA (also known as SiR-Halo) is a ChloroAlkane (CA) derivative of the Silicon Rhodamine (SiR) fluorophore. It emits light in the far-red, is fluorogenic and well suited for STED and SIM superresolution imaging. SiR-CA can be imaged with a standard Cy5 settings. It can be used for widefield, confocal, 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 SiR-CA (10 nmol, lyophilized).



SiR-CA structure

Properties

Absorbance maximum λ_{abs}	652 nm
Fluorescence maximum λ_{fl}	674 nm
Works on fixed cells?	yes
Quantity	10 nmol
Fluorescence lifetime (HT7 bound)	3.2 ns
MW	678.3 g/mol
STED depletion wavelength	775 nm
Shipping	room temperature
Storage	-20°C



SiR absorption (solid) and emission (dashed) spectra

Storage & Handling

Store the SiR-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 SiR-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 SiR-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.

Version 09.2024

Spirochrome AG – Chalberwidstrasse 4
8260 Stein am Rhein - Switzerland
Email: info@spirochrome.com
Website: www.spirochrome.com

1. Prepare DMSO stock solution. Add 50 µL of anhydrous DMSO to the SiR-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 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 SiR-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 SiR-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, SiR-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 SiR-CA labeled cells is best performed using standard Cy5 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

Spirochrome 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. Spirochrome products and product applications are covered by patents and patents pending.

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, Chalberwidstrasse 4, 8620 Stein am Rhein, Switzerland, Email: info@spirochrome.com