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Product information: SiR700-lysosome Kit (SC016)

Live Cell Fluorogenic Lysosome Labelling Probe

Introduction

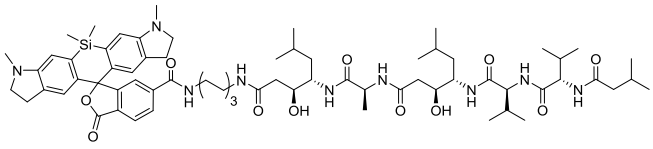
SiR700-lysosome is based on the silicon rhodamine (SiR) fluorophore analogue SiR700 and the cathepsin D binding peptide pepstatin A. SiR700-lysosome allows the labelling of lysosomes in live cells with high specificity and low background[1)]. The key features of SiR700-lysosome are i) far-red absorption and emission wavelengths, ii) cell permeability, iii) fluorogenic character and iv) compatibility with superresolution microscopy (STED & SIM). The unprecedented combination of those properties in a single probe put SiR700-lysosome at the leading edge of excellence.

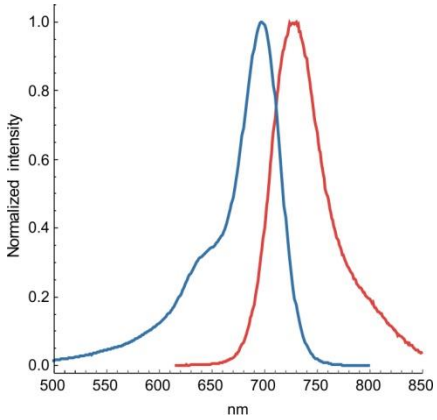
Storage & Handling

Store the compound below -20°C upon receipt. Prepare solutions of the compound using anhydrous DMSO. Keep solutions of the compound below -20°C after use. Vials should be allowed to warm to room temperature before opening. When stored properly, the compound should be stable for several 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.

Physical properties

λ_{abs}	689 nm
λ_{Em}	716 nm
ε_{max}	$1.0 \cdot 10^5$ $mol^{-1} \cdot cm^{-1}$
MW	1262.7 g/mol
MF	$C_{69}H_{103}N_9O_{11}Si$





Kit contents: 35 nmol SiR700-lysosome and 1 μmol verapamil

Labelling Protocol

Note: This protocol was optimized using human fibroblast cells adhering to coverslips and has been confirmed in other common cell lines. Recommendations for experimental protocols should be used as a starting point, and optimal labeling conditions for each cell type should be determined empirically. SiR700-lysosome is based on the cathepsin D inhibitor pepstatin A. It may therefore modify lysosome metabolism in living cells.

Prepare 1 mM stock solution. Dissolve the content of the vial of SiR700-lysosome in 35 µL of anhydrous DMSO to make a 1 mM stock solution. This solution should be stored at -20°C or below. **Do not divide the 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 should be stable for three months or more. If the concentration of the stock solution needs be accurately determined, dilute 1 µl of 1 mM stock solution in 99 µl of PBS containing 0.2 % SDS. After 15 minutes at room temperature, measure the absorbance at 689 nm. Calculate the concentration using the extinction coefficient given above.

Prepare staining solution. Dilute SiR700-lysosome to the desired concentration in cell culture medium (e.g. DMEM + 10% fetal bovine serum) and vortex briefly. Since staining efficiency can depend on the cell line, it is recommended to stain cells with 1 µM at the first attempt to quickly obtain a strong staining and then reduce the SiR700-lysosome concentration in further experiments until an optimal staining is achieved (see labelling concentration & incubation time table below). Some cell lines might express high levels of efflux pumps and are poorly stained by SiR700-lysosome. The addition of 10 µM verapamil, a broad spectrum efflux pump inhibitor, in the staining solution usually greatly improves the staining. See www.spirochrome.com/verapamil for more information on the use of verapamil with SiR-probes. Use only freshly made staining solution and do not use it multiple times.

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** ensuring that all the cells are covered with solution. Place the cells in the incubator at 37°C in a humidified atmosphere containing 5% CO_2 and observe the following table to determine labelling time as a function of probe concentration:

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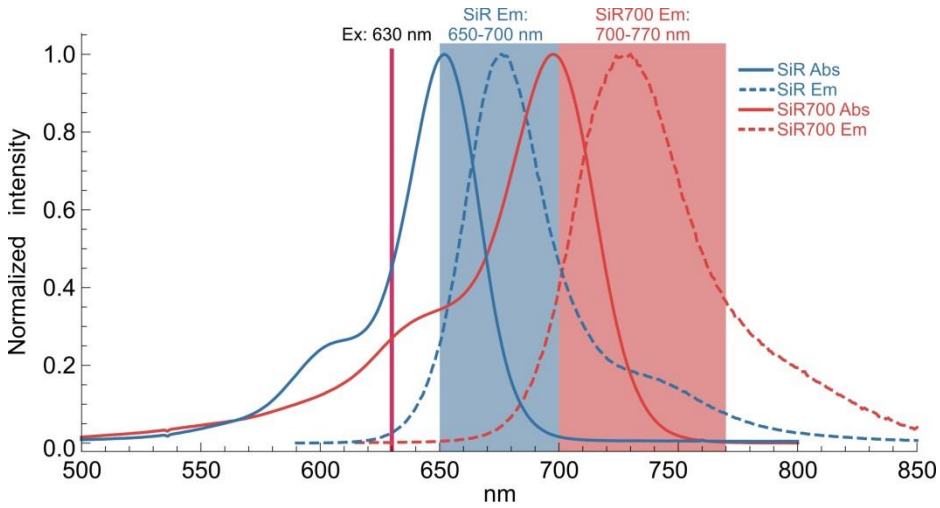
probe concentration (nM)	suggested labelling time (h)*
> 1000	0.5 - 1
500	3 - 4
200	4 - 6
< 100	6 - 12

* these labelling times were determined for human fibroblasts and may differ depending on the cell line used.

Cell imaging. Imaging of SiR700-lysosome 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 probe usually improves the signal to noise ratio. If time lapse imaging is performed, it is recommended to keep the concentration of probe as low as possible during the whole experiment to get a constant signal and to avoid interference of the probe. If cells were washed before imaging, the staining will last for a few hours.

Dual colour imaging SiR700-lysosome can be used together with spirochrome SiR-probes to perform dual colour imaging. SiR700-lysosome and the second SiR-probe (e.g. SiR-tubulin, SiR-DNA or SiR-lysosome) can be added simultaneously to the cells using the standard protocol above. A washing step is recommended to obtain the best signal to background ratio. Imaging is best performed using the following parameters: excitation using 630 to 640 nm light for both SiR700 and SiR; emission 650-700 nm (SiR channel) and 700-770 nm (SiR700 channel). A small bleed through of SiR into the SiR700 channel and vice versa may be observed. For more information and examples of dual colour imaging please visit our website www.spirochrome.com/dualcolour.



References:

1. Fluorogenic probes for multicolor imaging in living cells, G. Lukinavičius et al., *JACS*, (2016)

2. Fluorogenic probes for live-cell imaging of the cytoskeleton, G. Lukinavičius et al., *Nature Methods*, 11, 731–733 (2014)

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