

## Product information: SiR-lysosome Kit (SC012)

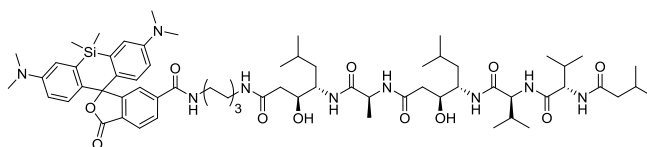
Live Cell Fluorogenic Lysosome Labelling Probe

### Introduction

SiR-lysosome is based on the fluorophore silicon rhodamine (SiR) and the cathepsin D binding peptide pepstatin A. SiR-lysosome allows the labelling of lysosomes in live cells with high specificity and low background<sup>1</sup>. The key features of SiR-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 SiR-lysosome at the leading edge of excellence.

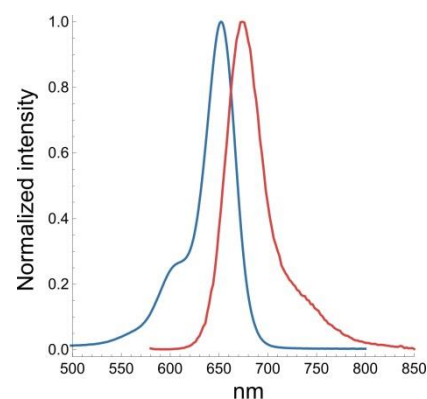
### Physical properties

$\lambda_{Abs}$	652 nm
$\lambda_{Em}$	674 nm
$\epsilon_{max}$	$1.0 \cdot 10^5 \text{ mol}^{-1} \cdot \text{cm}^{-1}$
MW	1237.7 g/mol
MF	$\text{C}_{67}\text{H}_{103}\text{N}_9\text{O}_{11}\text{Si}$



### Storage & Handling

Store the compound below  $-20^\circ\text{C}$  upon receipt. Prepare solutions of the compound using anhydrous DMSO. Keep solutions of the compound below  $-20^\circ\text{C}$  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.



**Kit contents:** 50 nmol SiR-lysosome and 1  $\mu\text{mol}$  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. SiR-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 SiR-lysosome in 50  $\mu\text{L}$  of anhydrous DMSO to make a 1 mM stock solution. This solution should be stored at  $-20^\circ\text{C}$  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 to be accurately determined, dilute 1  $\mu\text{L}$  of 1 mM stock solution in 99  $\mu\text{L}$  of PBS containing 0.2 % SDS. After 15 minutes at room temperature, measure the absorbance at 652 nm. Calculate the concentration using the extinction coefficient given above.

**Prepare staining solution.** Dilute SiR-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  $\mu\text{M}$  at the first attempt to quickly obtain a strong staining and then reduce the SiR-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 SiR-lysosome. The addition of 10  $\mu\text{M}$  verapamil, a broad spectrum efflux pump inhibitor, in the staining solution usually greatly improves the staining. See [www.spirochrome.com/verapamil](http://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  $37^\circ\text{C}$  in a humidified atmosphere containing 5%  $\text{CO}_2$  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

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

**Cell imaging.** Imaging of SiR-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 with lysosome metabolism. If cells were washed before imaging, the staining will last for a few hours.

#### 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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