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Product information: SiR700-tubulin Kit (CY-SC014)

Live Cell Fluorogenic Microtubule Labelling Probe

Introduction

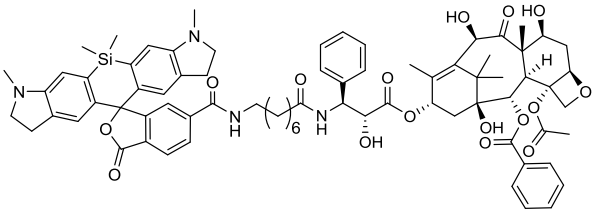
SiR700-tubulin is based on the silicon rhodamine (SiR) fluorophore analogue SiR700 and the microtubule binding drug Docetaxel. SiR700-tubulin allows the labelling of microtubules in live cells with high specificity and low background[1)]. The key features of SiR700-tubulin are i) far-red absorption and 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-tubulin at the leading edge of excellence.

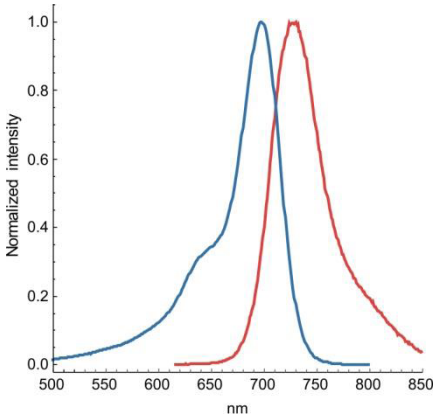
Storage & Handling

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

$_{abs}$	689 nm
$_{Em}$	716 nm
$\varepsilon_{689\ nm}$	$1.0 \cdot 10^5$ $mol^{-1} \cdot cm^{-1}$
MW	1327.6 g/mol
MF	$C_{75}H_{86}N_4O_{16}Si$





Kit contents: 35 nmol SiR700-tubulin 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-actin is based on the actin stabilizing drug jasplakinolide. It can therefore modify tubulin dynamics in living cells. Whereas interphase cells were only little affected by the probe, concentrations above 100 nM of SiR700-tubulin led to mitotic duration increase in cultured HeLa cells[1)]. If long term imaging experiments are planned where tubulin dynamics are critical, we recommend to keep the concentration of SiR700-tubulin equal or below 100 nM. For other purposes, using 0.5-1 M SiR700-tubulin for staining is recommended.

Prepare 1 mM stock solution. Dissolve the content of the vial of SiR700-tubulin 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 l of 1 mM stock solution in 99 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 SiR700-tubulin 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 M at the first attempt to quickly obtain a strong staining and then reduce the SiR700-tubulin 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 may therefore be poorly stained by SiR700-tubulin. The addition of 10 M verapamil, a broad spectrum efflux pump inhibitor, in the staining solution usually greatly improves the staining. Use only freshly made staining solution and do not use it multiple times.

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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:

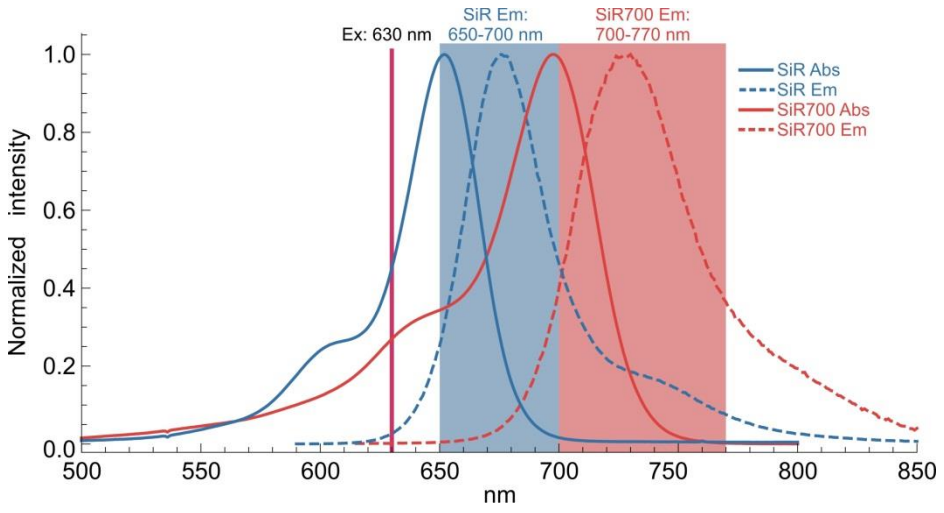
probe concentration (nM)	suggested labelling time (h)*
> 1000	0.5 - 1
500	3 - 4
200	4 - 6
< 100	6 - 12

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

Note: SiR700-tubulin does not stain paraformaldehyde (PFA) and methanol fixed cells as these fixation methods alter the probe binding site on microtubules.

Cell imaging. Imaging of SiR700-tubulin can be performed using standard Cy5 settings, however recording fluorescence emission between 700 and 800 nm provides a better signal. After labelling, the live cells can be immediately imaged without the need for washing steps. Optionally, a simple washing step consisting of replacing 1-3 times 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 equal or below 100 nM during the whole experiment to get a constant signal and to avoid interference of the probe with tubulin dynamics (reduced cell proliferation). If cells were washed before imaging, the staining will last for a few hours.

Dual colour imaging. SiR700-tubulin can be used together with spirochrome SiR-probes to perform dual colour imaging. SiR700-tubulin and the second SiR-probe (e.g. SiR-actin, 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 live-cell imaging of the cytoskeleton, G. Lukinavičius et al., *Nature Methods*, 11, 731–733 (2014)

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

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