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Product information: Cytoskeleton Kit (SC006)

Live Cell Fluorogenic Microtubule Labelling Probe

Kit contains 1x 50 nmol SiR-actin 1x 50 nmol SiR-tubulin and 1x 1 μmol verapamil

SiR-actin usage:

Introduction

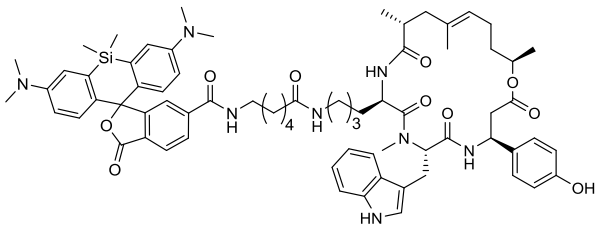
SiR-actin is based on the fluorophore silicon rhodamine (SiR) and the actin binding natural product jasplakinolide. Sir-actin allows the labelling of F-actin in live cells with high specificity and low background[1)]. The key features of SiR-actin are i) far-red absorption and emission wavelengths, ii) 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-actin at the leading edge of excellence.

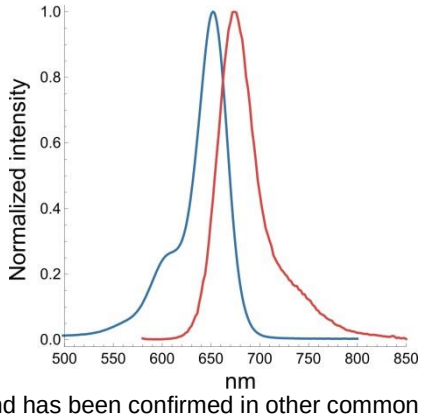
Storage & Handling

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}	652 nm
λ_{Em}	674 nm
$\varepsilon_{652\ nm}$	$1.0 \cdot 10^5$ $mol^{-1} \cdot cm^{-1}$
MW	1241.6 g/mol
MF	$C_{71}H_{88}N_8O_{10}Si$





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-actin is based on the actin stabilizing drug jasplakinolide. It can therefore modify actin dynamics in living cells. While mitotic duration remained unchanged at concentrations up to 3 μM SiR-actin, concentrations above 100 nM led to reduced cell proliferation index in cultured HeLa cells[1)]. If long term imaging experiments are planned where actin dynamics are critical, we recommend to keep the concentration of SiR-actin equal or below 100 nM. For other purposes, using 1 μM SiR-actin for staining is recommended.

Prepare 1 mM stock solution. Dissolve the content of the vial of SiR-actin in 50 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 652 nm. Calculate the concentration using the extinction coefficient given above.

Prepare staining solution. Dilute SiR-actin 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 SiR-actin 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-actin. 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.

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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: SiR-actin stains F-actin in paraformaldehyde (PFA) fixed cells as efficiently as phalloidin using standard phalloidin staining protocol.

Cell imaging. Imaging of SiR-actin 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 equal or below 100 nM during the whole experiment to get a constant signal and to avoid interference of the probe with actin dynamics (reduced cell proliferation). If cells were washed before imaging, the staining will last for a few hours.

SiR-Tubulin usage:

Introduction

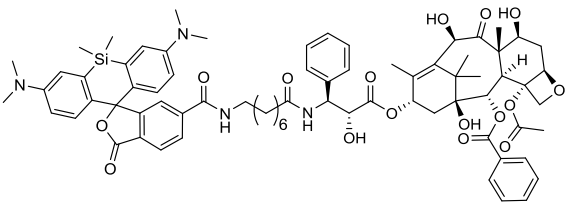
SiR-tubulin is based on the fluorophore silicon rhodamine (SiR) and the microtubule binding drug Docetaxel. Sir-tubulin allows the labelling of microtubules in live cells with high specificity and low background[1)]. The key features of SiR-tubulin are i) far-red absorption and emission wavelengths,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

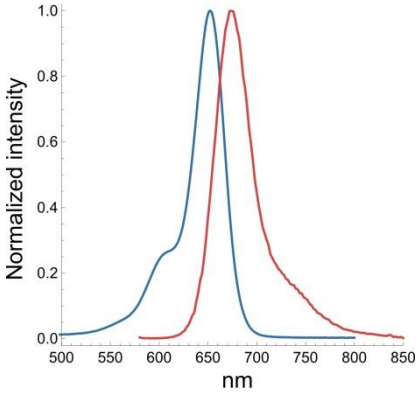
Storage & Handling

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

λ_{abs}	652 nm
λ_{Em}	674 nm
$\varepsilon_{652\ nm}$	$1.0 \cdot 10^5$ $mol^{-1} \cdot cm^{-1}$
MW	1303.6 g/mol
MF	$C_{73}H_{86}N_4O_{16}Si$





Labelling Protocol

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

Prepare 1 mM stock solution. Dissolve the content of the vial of SiR-tubulin in 50 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 652 nm. Calculate the concentration using the extinction coefficient given above.

Prepare staining solution. Dilute SiR-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 μM at the first attempt to quickly obtain a strong staining and then reduce the SiR-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 are poorly stained by SiR-tubulin. 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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500	3 - 4
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< 100	6 - 12

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

Important note: SiR-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 SiR-tubulin 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 equal or below 100 nM during the whole experiment to get a constant signal and to avoid interference of the probe with microtubule dynamics (prolonged mitotic duration and reduced cell proliferation). If cells were washed before imaging, the staining will last for a few hours.

References:

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

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