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# *Product information:* SiR-DNA (SC007)

Live Cell Fluorogenic DNA Labelling Probe

## Introduction

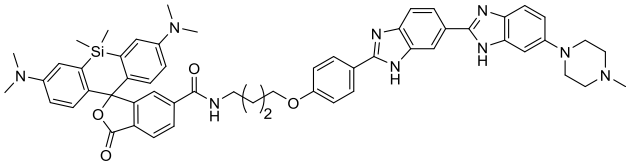
SiR-DNA is based on the fluorophore silicon rhodamine (SiR) and the DNA minor groove binder bisbenzimide (Hoechst). Sir-DNA allows the labelling of DNA in live cells with high specificity and low background[1)]. The key features of SiR-DNA are i) far-red absorption and emission wavelengths, ii) cell permeability, iii) fluorogenic character and iv) compatibility with superresolution microscopy (STED & SIM). The unprecedented combination of those properties in a single probe put SiR-DNA at the leading edge of excellence.

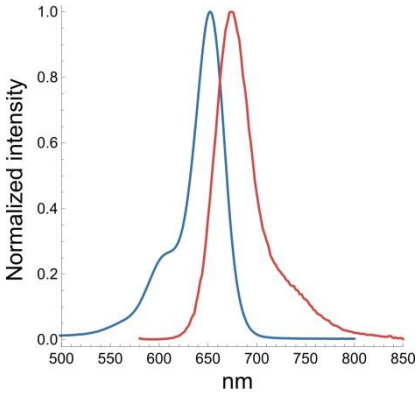
## Storage & Handling

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

| | |
|---|---|
| $\lambda_{abs}$ | 652 nm |
| $\lambda_{Em}$ | 674 nm |
| $\varepsilon_{652\ nm}$ | $1.0 \cdot 10^5$ $mol^{-1} \cdot cm^{-1}$ |
| MW | 950.2 g/mol |
| MF | $C_{56}H_{59}N_9O_4Si$ |






## 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-DNA is based on the DNA minor groove binding molecule bisbenzimide. It might therefore modify DNA metabolism in living cells. Mitotic duration and chromosome missegregation remained unchanged at concentrations up to 10 μM SiR-DNA. Cell proliferation index in cultured HeLa cells was not affected up to a concentration of 25 μM SiR-DNA [1)]. However, if long term imaging experiments are planned, we recommend to keep the concentration of SiR-DNA equal or below 1 μM. For other purposes, using 1-3 μM SiR-DNA for staining is recommended.

**Prepare 1 mM stock solution**. Dissolve the content of the vial of SiR-DNA 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-DNA 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 3 μM at the first attempt to quickly obtain a strong staining and then reduce the SiR-DNA 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 be less effeciently stained by SiR-DNA. The addition of 1-10 μM verapamil, a broad spectrum efflux pump inhibitor, in the staining solution usually greatly improves the staining (for more information, see http://spirochrome.com/verapamil/ . 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



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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 | 2 – 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-DNA stains DNA in paraformaldehyde (PFA) and methanol fixed cells.**

**Cell imaging.** Imaging of SiR-DNA 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 may improve the signal to noise ratio. If time lapse imaging is performed, it is recommended to keep the concentration of probe equal or below 1 μM in the imaging medium during the whole experiment to get a constant signal and to avoid interference of the probe with DNA metabolism. If cells were washed before imaging, the staining will last for a few hours.

**References:**

**1.** Lukinavicius, G. *et al.* SiR-Hoechst is a far-red DNA stain for live-cell nanoscopy. *Nat. Commun.* 6:8497 doi: 10.1038/ncomms9497 (2015).