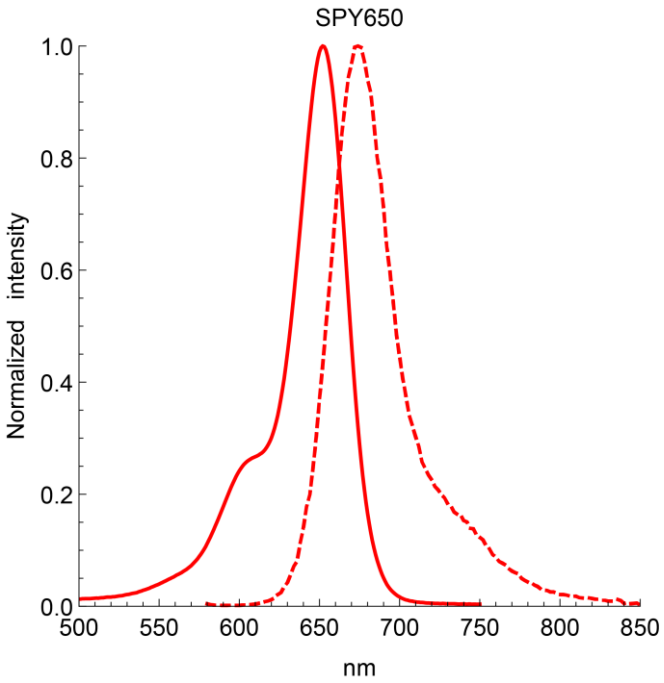
Spirochrome AG - Postfach 213
8260 Stein am Rhein - Switzerland
Email: info@spirochrome.com
Website: www.spirochrome.com

Product information: SPY650-DNA (SC501)

Live Cell Fluorogenic DNA Labelling Probe

Introduction

SPY650-DNA is a bright far-red & non toxic live cell nuclear stain based on our SPY™ dyes series. Its optimized structure allows quick labeling of DNA in live & fixed cells with high specificity and very low background. SPY650-DNA stains the nuclei of live or fixed cells without the need for genetic manipulation or overexpression of fluorescent proteins. Its emission in the far red minimizes phototoxicity and sample autofluorescence. SPY650-DNA enables multicolor imaging with SPY505, SPY555, SPY595, SPY700, GFP or m-cherry. SPY650-DNA can be imaged with standard Cy5 filtersets. It can be used for widefield, confocal, SIM or STED imaging in living or fixed cells and tissue. Contains 1 vial of SPY650-DNA (lyophilized).



Probe Properties

Absorbance maximum λ_{abs}	652 nm
Fluorescence maximum λ_{fl}	674 nm
Works on fixed cells?	yes, PFA and methanol
Probe quantity	100 stainings*
Fluorescence lifetime	3.0 ns
STED depletion wavelength	775 nm
Shipping	room temperature
Storage	-20°C

Storage & Handling

Store the probe at -20°C or below upon receipt. The lyophilized probe is stable for >1 week at room temperature and for >12 months at -20°C. Reconstitute SPY650-DNA using anhydrous DMSO. We recommend to use newly or freshly opened and anhydrous DMSO to prepare the 1000x stock solution. In contact with air and moisture, DMSO produces decay products which can strongly reduce the shelf life of the probe in solution, even at -20°C. Keep the 1000x stock solution of the probe below -20°C after use. Vials should be allowed to warm to room temperature before opening. When reconstituted and stored properly, the 1000x stock solution is stable for 3 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.

Labelling Protocol

Note: This protocol was optimized using HeLa cells adhering to coverslips and has been confirmed in other common cell lines. Recommendations in this protocol should be used as a starting point, and optimal labeling conditions for each cell type should be determined empirically. SPY650-DNA is based on the DNA minor groove binding molecule bisbenzimide. It may modify DNA metabolism in living cells at high doses. Therefore the recommended staining dilution is 1000 fold or more if long term (>12h) imaging experiments are planned. For all other purposes, 1000 fold dilution SPY650DNA for staining is recommended.

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1. Prepare 1000x stock solution. Add 50 µL of anhydrous DMSO to the SPY650-DNA vial to prepare the 1000x stock solution. We recommend to use newly or freshly opened and anhydrous DMSO to prepare the 1000x stock solution. In contact with air and moisture, DMSO produces decay products which can strongly reduce the shelf life of the probe in solution, even at -20°C. At this stage, the solution can be colored or not, this has no influence on the performance of the probe. After use, this solution should be stored at -20°C or below. Do not divide the 1000x stock solution into small aliquots, they will decay faster and the probe is not altered by multiple freeze-thaw cycles. When stored properly, this stock solution is stable for 3 months.

2. Prepare the staining solution. Dilute SPY650-DNA to 1x in your usual cell culture medium (e.g. DMEM + 10% fetal bovine serum) and vortex briefly. If the dilution is not performed in a single step, please use DMSO to prepare the intermediate dilution as using aqueous buffers to prepare the intermediate dilution will lead to the formation of probe aggregates. Proceed quickly to step 3. Since staining efficiency can depend on the cell line, it is recommended to stain cells with 1000x dilution at the first attempt and then optimize the SPY650-DNA dilution factor in further experiments until an optimal staining is achieved (see labelling concentration & incubation time table below). Use only freshly made staining solution, and do not use it multiple times.

3. 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** freshly prepared under step 2 ensuring that all the cells are covered with the 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:

Dilution factor	suggested labelling time (h)**
1000 or less	1
2000	2
>2000	4

Note: SPY650-DNA stains DNA in paraformaldehyde (PFA) and methanol fixed cells.

4. Cell imaging. Imaging of SPY650-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 probe in the imaging medium during the whole experiment to get a constant signal. If the cells were washed before imaging, the staining will last for a few hours. Please note that SPY650-DNA may be excited by 360-390 nm light and produce some fluorescence at ca. 450 nm due to fluorescence of the DNA binding moiety of the probe.

* Based on the following conditions: 0.5 ml staining solution / staining experiment with 1x probe concentration. The number of staining experiments can be further increased by reducing volume or probe concentration.

** These labelling times were determined for HeLa cells and may differ depending on the cell line used.

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