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Product information: SPY700-tubulin (CY-SC603)

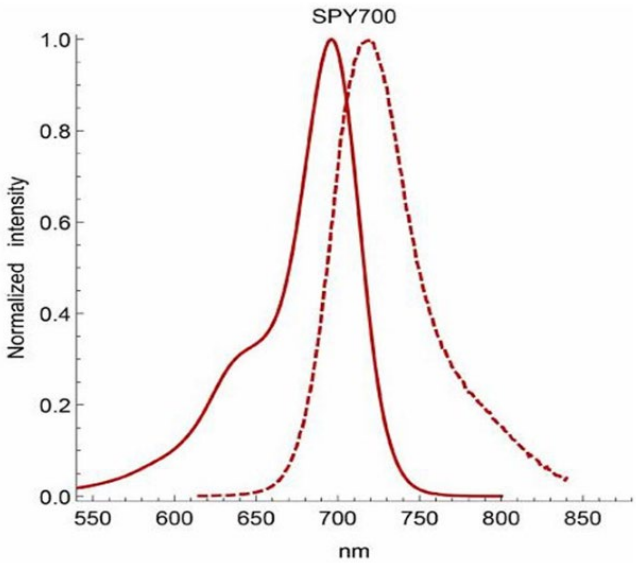
Live Cell Fluorogenic microtubule Labelling Probe

Introduction

SPY700-tubulin is a bright, far-red & non toxic live cell microtubule stain based on our SPY™ dyes series. Its optimized structure allows quick labeling of microtubules in live cells with high specificity and very low background. SPY700-tubulin stains microtubules in live cells without the need for genetic manipulation or overexpression of fluorescent proteins. Its absorbance and emission spectra are similar to Cy5.5. SPY700-tubulin enables multicolor imaging with SPY505, SPY555, SPY595, SPY650, GFP or m-cherry. SPY700-tubulin can be imaged with standard Cy5.5 filter sets. It can be used for widefield, confocal, SIM or STED imaging in living cells and tissue. Contains 1 vial of SPY700-tubulin (lyophilized).

Probe Properties

Absorbance maximum λ_{abs}	696 nm
Fluorescence maximum λ_{fl}	718 nm
Works on fixed cells?	no
Probe quantity	100 stainings*
Fluorescence lifetime	2.2 ns
STED depletion wavelength	>800 nm
Shipping	room temperature
Storage	-20°C



Storage & Handling

Store the kit 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 SPY700-tubulin using anhydrous DMSO. We recommend using 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 should 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. SPY700-tubulin is based on a fluorescent taxol derivative. It may therefore modify microtubule metabolism in living cells at high concentration. 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 SPY700-tubulin for staining is recommended.

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1. Prepare 1000x stock solution. Add 50 µL of anhydrous DMSO to the SPY700-tubulin 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 SPY700-tubulin 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 SPY700-tubulin 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

4. Cell imaging. Imaging of SPY700-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 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.

* 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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