Product information: SPY555-tubulin (SC 203)

Live Cell Fluorogenic microtubule Labelling Probe

Introduction

SPY555-tubulin is a bright & 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. SPY555-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 tetramethylrhodamine (TMR). SPY555-tubulin enables multicolor imaging with SPY505, SPY595, SPY650, SPY700, SiR and GFP. SPY555-tubulin can be imaged with standard TMR or Cy3 filtersets. It can be used for widefield, confocal, SIM or STED imaging in living cells and tissue. Contains 1 vial of SPY555-tubulin (lyophilized).

Probe Properties

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance maximum λ&lt;sub&gt;abs&lt;/sub&gt;</td>
<td>555 nm</td>
</tr>
<tr>
<td>Fluorescence maximum λ&lt;sub&gt;fl&lt;/sub&gt;</td>
<td>580 nm</td>
</tr>
<tr>
<td>Works on fixed cells?</td>
<td>no</td>
</tr>
<tr>
<td>Probe quantity</td>
<td>100 stainings*</td>
</tr>
<tr>
<td>Fluorescence lifetime</td>
<td>2.4 ns</td>
</tr>
<tr>
<td>STED depletion wavelength</td>
<td>660 or 775 nm</td>
</tr>
<tr>
<td>Shipping</td>
<td>room temperature</td>
</tr>
<tr>
<td>Storage</td>
<td>-20°C</td>
</tr>
</tbody>
</table>

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 SPY555-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 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. SPY555-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 SPY555-tubulin for staining is recommended.

1. Prepare 1000x stock solution. Add 50 μL of anhydrous DMSO to the SPY555-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
After labelling, the live cells Spirochrome products and product applications are covered by
of www.spirochrome.com

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% CO₂ and observe the following table to determine labelling time as a function of probe concentration:

<table>
<thead>
<tr>
<th>Dilution factor</th>
<th>suggested labelling time (h)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 or less</td>
<td>1</td>
</tr>
<tr>
<td>2000</td>
<td>2</td>
</tr>
<tr>
<td>&gt;2000</td>
<td>4</td>
</tr>
</tbody>
</table>

4. Cell imaging. Imaging of SPY555-tubulin is best performed using standard TMR or Cy3 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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