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# *Product information:* CenSpark650 SC525

Live Cell Fluorogenic Centriole and Cilia Probe

### Introduction

CenSpark650 is a bright, far red & non-toxic live cell centriole and cilia probe based on our proprietary Silicon Rhodamine (SiR) dye. Its optimized structure allows a selective labeling of centrioles and cilia in live cells and tissues with high specificity and low background. CenSpark650 stains centrioles and cilia in live cells without the need for genetic manipulation or overexpression of fluorescent proteins. Its absorbance and emission spectra are similar to Cy5. CenSpark650 enables multicolor imaging with SPY505, SPY555, SPY595, SPY700, GFP or m-cherry. CenSpark650 can be imaged with standard Cy5 filtersets. It can be used for, confocal, SIM or STED imaging in living cells and tissue. Contains 1 vial of CenSpark650 (lyophilized).

### 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 CenSpark650 using anhydrous DMSO. We recommend using newly or freshly opened and anhydrous DMSO to prepare the 1000x stock solution. Store 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.

### Probe Properties

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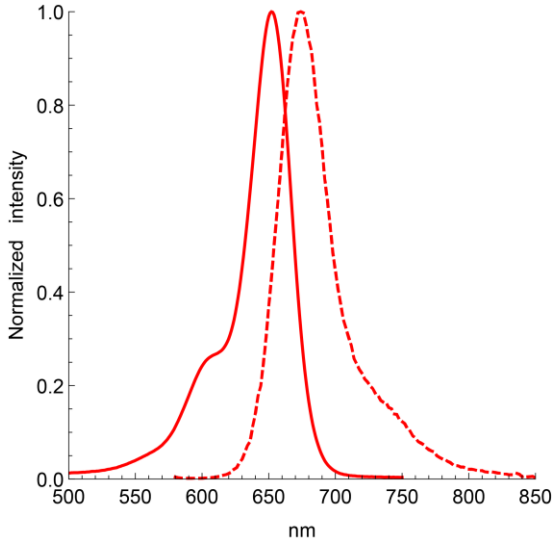
|---|---|
| **Absorbance maximum $\lambda_{abs}$** | 652 nm |
| **Fluorescence maximum $\lambda_{fl}$** | 674 nm |
| **Works on fixed cells?** | Yes, GA and Acetone fixation |
| **Probe quantity** | 100 stainings* |
| **Fluorescence lifetime** | 3.0 ns |
| **STED depletion wavelength** | 775 nm |
| **Shipping** | room temperature |
| **Storage** | -20°C |




### Labelling Protocol

**Important Note:**

This protocol was optimized and validated using HeLa, or U2OS cells adhering to coated glass or polymer dishes. For other 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.

**1. Prepare 1000x stock solution**. Add 50 µL of anhydrous DMSO to the CenSpark650 vial to prepare the 1000x stock solution (DMSO stock concentration is 1 mM). 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. 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 may 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 CenSpark650 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 may 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



Version 05.2026

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then optimize the CenSpark650 dilution factor in further experiments until an optimal staining is achieved. 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% $CO_2$ for 1-2h**.

**4. Cell imaging.** Imaging of CenSpark650 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.

* 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.

** The recommended labelling time weas determined for 2D cultured cells and may differ slightly depending on the cell line used or the sample type (e.g. spheroids, organoids or tissue).