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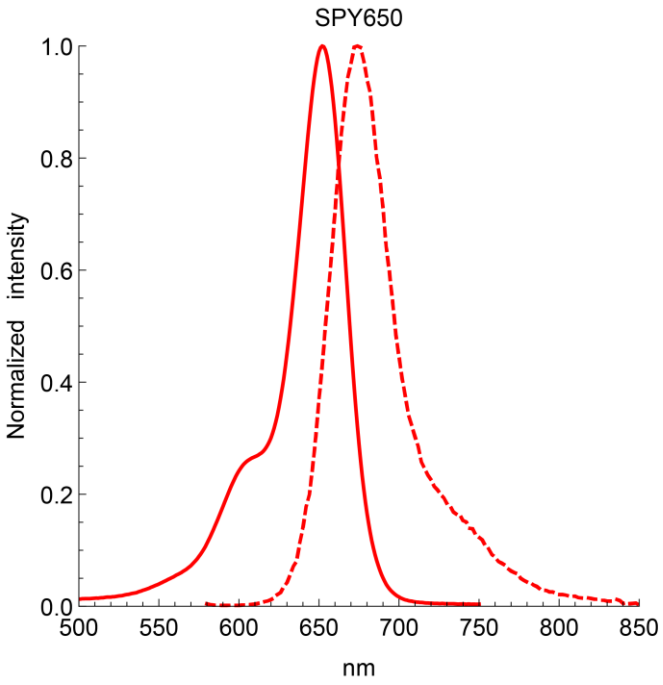
Product information: SPY650-FastAct_X (SC502)

Live Cell Fluorogenic F-actin Labelling Probe

Introduction

SPY650-FastAct_X is a bright, far red, fluorogenic & non toxic F-actin stain based on our SPY™ dyes series. SPY650-FastAct_X's carefully optimized actin ligand allows to label F-actin in live cells with very high specificity. SPY650-FastAct_X can label very fast actin dynamics but lacks undesired F-actin stabilization side effect. The probe does not require any genetic manipulation, transfection or overexpression of fluorescent proteins. SPY650-FastAct_X is based on our bright, photostable & far red SPY650 fluorophore which is far superior than fluorescent proteins. SPY650-FastAct_X enables multicolor imaging with SPY505, SPY555, SPY595, SPY700, GFP or m-cherry. SPY650-FastAct_X can be imaged with standard Cy5 filterset. It can be used for widefield, confocal, SIM or STED imaging of living cells and tissue. Contains 1 vial of SPY650-FastAct_X (lyophilized).



Probe Properties

Absorbance maximum λ_{abs}	652 nm
Fluorescence maximum λ_{fl}	674 nm
Works on fixed cells?	No
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-FastAct_X 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

Important Notes:

a) This protocol was optimized and validated using HeLa 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.

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b) SPY650-FastAct_X is mildly fluorogenic and is best imaged by microscopes that are capable of out of focus light exclusion, such as confocal, spinning disk, STED or light sheet microscopes. SPY650-FastAct_X will yield a higher background signal using widefield based microscopy techniques.

1. Prepare 1000x stock solution. Add 50 µL of anhydrous DMSO to the SPY650-FastAct_X 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 many freeze-thaw cycles. When stored properly, this stock solution is stable for 3 months.

2. Prepare the staining solution. Dilute SPY650-FastAct_X 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-FastAct_X 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	1-2
2000	4
>2000	6

After the labelling, do not wash out the cells, the probe is fluorogenic.

4. Cell imaging. Imaging of SPY650-FastAct_X is best performed using standard Cy5 settings. After labelling, the live cells can be immediately imaged. Washing steps are not recommended with of SPY650-FastAct_X.

* 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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