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

# *Product information:* SpyRho 555 (SC018)

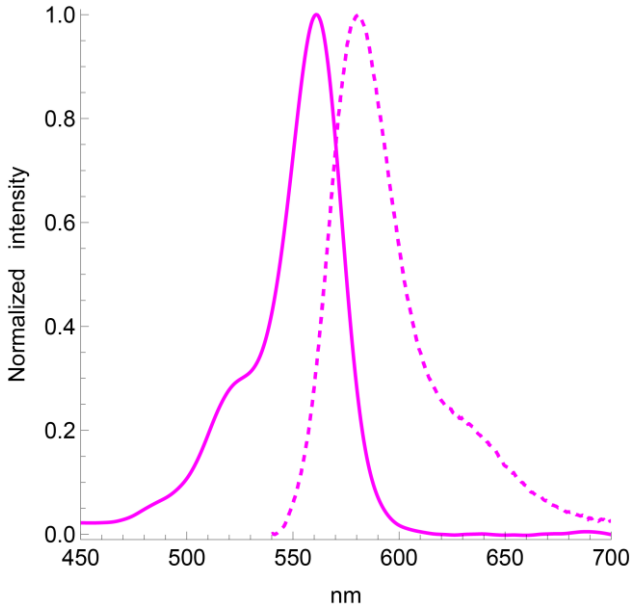
Fluorogenic dye binding to RhoBAST RNA aptamer for live cell RNA imaging

### Introduction

SpyRho 555 is a fluorogenic dye ligand for the RNA aptamer RhoBAST
SpyRho 555 was developed by the lab of Murat Sunbul in Heidelberg University[1)]. SpyRho 555 is cell permeable, highly fluorogenic and photostable. With its high affinity and fast on/off binding kinetics, it allows overcoming the main issues of live cell RNA imaging. SpyRho 555 is highly suited for widefield, confocal, STED or singlmolecule localization microscopy. SpyRho 555 can be imaged with standard TMR or Cy3 filtersets. It can be used in living or fixed cells. Contains 1 vial of SpyRho 555 (lyophilized).

### Properties

| | |
|---|---|
| **Absorbance maximum $\lambda_{abs}$** | **562 nm** |
| **Fluorescence maximum $\lambda_{fl}$** | 581 nm |
| **Works on fixed cells?** | Yes |
| **Quantity** | 100 stainings* |
| **$\Phi_F$ (RNA bound)** | 0.95 |
| **Kd to RhoBAST aptamer** | 34 nM |
| **STED depletion wavelength** | 660 nm |
| **MW** | 492.5 g/mol |
| **Shipping** | room temperature |
| **Storage** | -20°C |



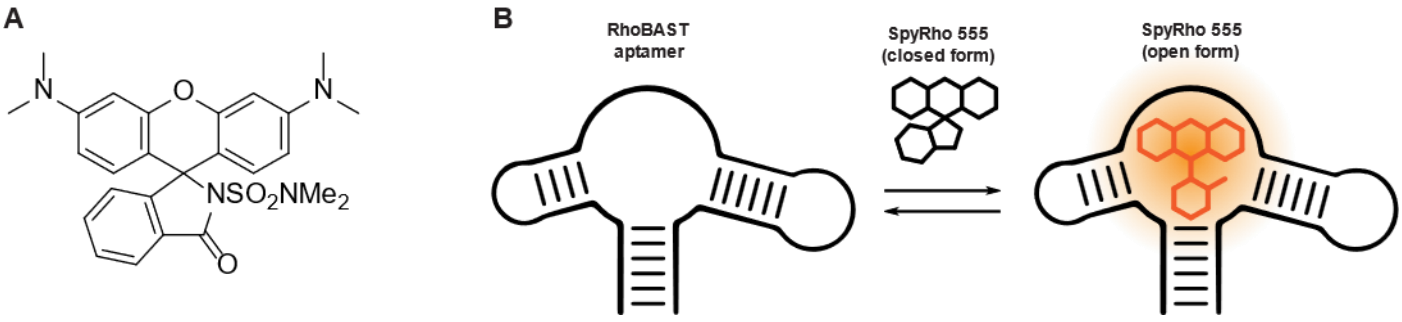




Figure 1. **A** Chemical structure of SpyRho 555. **B** SpyRho 555 reversibly binds to RhoBAST aptamer with fast kinetics. Unbound SpyRho 555 exists predominantly in the closed form and is not fluorescent. Upon binding to RhoBAST aptamer, SpyRho 555 switches to the open form and becomes highly fluorescent.

### Storage & Handling

Store the SpyRho 555 at -20°C or below upon receipt. Lyophilized SpyRho 555 is stable for >1 week at room temperature and for at least 6 months at -20°C. Reconstitute SpyRho 555 using anhydrous DMSO. We recommend to use newly or freshly opened and anhydrous DMSO to prepare the stock solution. In contact with air and moisture, DMSO produces decay products which can strongly reduce the shelf life of SpyRho 555 in solution, even at -20°C. Keep the stock solution of the SpyRho 555 below -20°C after use. Vials should be allowed to warm to room temperature before opening. When reconstituted and stored properly, the stock solution is stable for up to 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.



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**Labelling Protocol**

**Note:** 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 DMSO stock solution**. Add 50 µL of anhydrous DMSO to the SpyRho 555 vial to prepare a 1000x stock solution (SpyRho 555 concentration: 100 µM). We recommend to use newly or freshly opened and anhydrous DMSO to prepare the DMSO stock solution. In contact with air and moisture, DMSO produces decay products which can strongly reduce the shelf life of the SpyRho 555 in solution, even at -20°C. At this stage, the solution can be colored or not, this has no influence on the performance of SpyRho 555. After use, this solution should be stored at -20°C or below. Do not divide the DMSO stock solution into small aliquots, they will decay faster and 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 the SpyRho 555 DMSO stock solution 1:1000 (final concentration 100 nM) in your usual cell culture medium (e.g. DMEM + 10% fetal bovine serum) and vortex briefly. Proceed quickly to step 3. 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 aggregates. Since staining efficiency can depend on the cell line, it is recommended to stain cells with 1:1000 dilution at the first attempt and then optimize the dilution factor in further experiments until an optimal staining is achieved. The usual range of SpyRho 555 concentration for live cell labelling is 1:1000 (100 nM) for widefield, Confocal, SIM or STED microscopy and 1:2000 (50 nM) or below for single molecule localization microscopy. Use only freshly made staining solution, and do not use it multiple times.

**3. Cell preparation and staining.** Grow cells transiently transfected or stably expressing a RhoBAST aptamer containing RNA on coverslips, glass bottom dish or glass bottom multi-well plates. When cells have reached the desired density or RNA expression levels, 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 30 minutes to 1h.

**4. Cell imaging.** Imaging of SpyRho 555 labeled cells is best performed using standard TMR or Cy3 settings. After labelling, the live cells can be immediately imaged without the need for washing steps. As the Binding of SpyRho 555 to ist cognate RhoBAST aptamer is highly dynamic and SpyRho is highly fluorogenic, we do not recommend a washing step after the staining in order to keep a strong signal over time.

* 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.

1. Daniel Englert et al. Fast-exchanging spirocyclic rhodamine probes for aptamer-based super-resolution RNA imaging" bioRxiv 2022.10.24.513449 https://doi.org/10.1101/2022.10.24.513449