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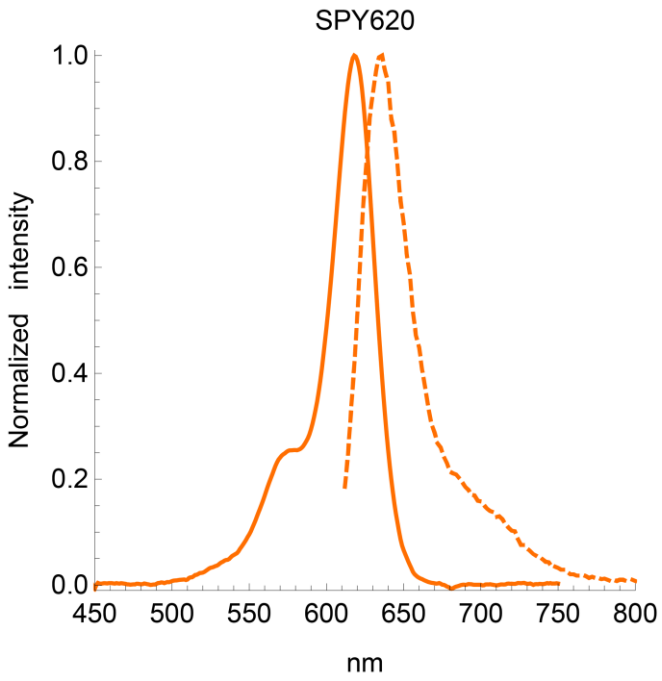
Product information: SPY620-BG (SC404)

SPY620-benzylguanine derivative for self-labelling tag staining

Introduction

Benzylguanine (BG) is the substrate of the self-labeling tag SNAP-tag™*. Upon reaction with a BG derivative, SNAP-tag™* forms a covalent bond with the substrate and releases guanine. It allows to permanently attach a fluorescent label to any protein of interest (POI) expressed as SNAP-tag™* fusion.
SPY620-BG is the benzylguanine derivative of our SPY620 fluorophore. It emits light in the red part of the spectrum, it is fluorogenic and well suited for STED and SIM superresolution imaging. SPY620-tubulin can be imaged with e.g. 610/20 nm and 650/40 nm excitation and emission filters respectively. It can be used for widefield, confocal, SIM or STED imaging in living or fixed cells and tissue. Contains 1 vial of SPY620-BG (35 nmol, lyophilized).



Properties

Absorbance maximum λ_{abs}	619 nm
Fluorescence maximum λ_{fl}	636 nm
Works on fixed cells?	yes
Quantity	35 mnol
Fluorescence lifetime	2.9 ns
STED depletion wavelength	775 nm
Shipping	room temperature
Storage	-20°C

Storage & Handling

Store the BG-substrate at -20°C or below upon receipt. The lyophilized BG-substrate is stable for >1 week at room temperature and for at least 6 months at -20°C. Reconstitute SPY620-BG 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 the BG-substrate in solution, even at -20°C. Keep the stock solution of the BG-substrate 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.

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.

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1. Prepare DMSO stock solution. Add 35 µL of anhydrous DMSO to the BG-substrate vial to prepare a 1 mM stock solution. 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 BG-substrate 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 BG-substrate. 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 the BG-substrate 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 BG-substrate DMSO stock solution 1:500 (final concentration 2 uM) in your usual cell culture medium (e.g. DMEM + 10% fetal bovine serum) and vortex briefly. For best performance serum or 0.5% BSA should be present in the cell culture medium. 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 will lead to the formation of BG-substrate aggregates. Since staining efficiency can depend on the cell line, it is recommended to stain cells with 1:500 dilution at the first attempt and then optimize the dilution factor in further experiments until an optimal staining is achieved. The usual range of BG-substrate concentration for live cell labelling is 1-10 uM. 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 SNAP-tag™* fusion-protein on coverslips, glass bottom dish or glass bottom multi-well plates as usual. When cells have reached the desired density or expression level of the SNAP-tag™* fusion-protein, 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.

Note: Before imaging, SPY620-BG stained cells can be fixed by any fixation method after the labelling step is completed. Additional immunolabeling or probe labeling can be performed after the fixation step using standard protocols.

4. Cell imaging. Imaging of SPY620-tubulin can be performed using e.g. 610/20 nm and 650/40 nm excitation and emission filters respectively. 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 BG-substrate may improve the signal to noise ratio. If the live cells were washed before imaging, the staining will last depending on your SNAP-tag™* fusion protein turnover rate.