GORYO CHEMICAL

For research use only

Updated: September 25, 2019

SaraFluor™ 650B-NHS

Table 1. Product information

Catalog no.	Product name	Amount	Storage upon receipt	Stability
A208-01	SaraFluor 650B-NHS	100 µg	≤−20°C, keep desiccated and protected from light.	1 year (when unopened and stored as described.)

1. About SaraFluor 650B-NHS

SaraFluor 650B-NHS is a fluorescent probe for super-resolution microscopy. Since this probe blinks spontaneously under physiological conditions, it can be used for single molecule localization microscopy using microscopes for PALM and STORM. For the observation, neither activation laser irradiation, nor additions of thiols/reducing reagents are required.

SaraFluor 650B-NHS forms a covalent bond with primary amines in antibodies, other proteins, or other molecules. Condensing reagents are not necessary for the reaction. This manual describes a protocol for conjugating SaraFluor 650B-NHS to about 5 mg of IgG antibody.

Table 2. Properties of SaraFluor 650B determined in 0.1M citrate buffer (pH 3.5).

λ_{ex} (nm)	λ_{em} (nm)	ε ($M^{-1}cm^{-1}$)
654	669	1.2×10^5

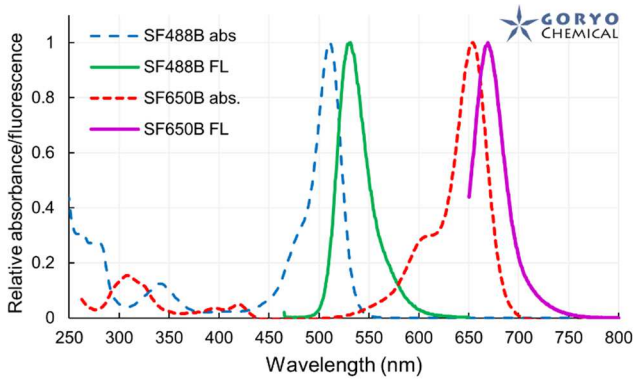
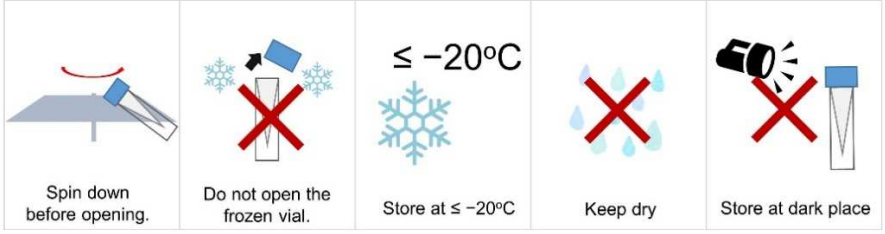


Fig. 1. Excitation (red) and emission (magenta) of SaraFluor 650B-NHS conjugated with a protein. The spectrum was measured in 0.1 M citrate buffer (pH 3.5).

■ Storage

This product is shipped as a solid in a nitrogen gas-filled vial. Upon receipt, store the product desiccated and protected from light at ≤ −20°C. Dissolve the reagents to DMSO just before the use. We provide no warranty for the reagents which was stored as a solution.



2. Protocol for IgG labeling

■ Materials required but not provided

・Anhydrous dimethyl sulfoxide (DMSO, anhydrous)
・Labeling buffer (0.1 M $NaHCO_3$, pH 8.4)
・Elution buffer (PBS pH 7.4, or appropriate buffer for the target protein)
・Centrifugal filters (e.g. Pall Nanosep or Amicon Ultra, with MWCO of 10kDa)
・Gel filtration column (e. g. NAP-5, GE Healthcare)
・Blocking solution (10 g/mL BSA in PBS)

■ Preparation of the reagent

1. SaraFluor 650B-NHS is pale blue solid, but it could be invisible because of its small amount. Warm the vial to the room temperature before opening the cap to prevent moisture absorption. Centrifuge before opening the cap to prevent the solid adhering to the cap. Add 18 µL of anhydrous DMSO to one vial to prepare 10 mM stock solution. Dissolve the solid completely through careful pipetting.
2. Use the solution immediately. Reactivity of NHS to amines is reduced by moisture absorption.

■ Procedure for labeling reaction

1. Rinse the centrifugal filters with PBS, centrifuge, and remove the PBS.
2. Remove the reservation buffer from the gel filtration column, add blocking solution, close the outlet of the column, and incubate for 15 min at room temperature (RT). Then open the outlet and elute with > ×10 volumes of the column to remove BSA. This blocking may increase the final protein yield.
3. Condense the target IgG to about ×10 concentration of the original solution using a centrifugal filter by following the instructions of the filter. Add the labeling buffer to the original volume and mix well. Centrifuge the solution again to obtain >1.5 mg/mL protein solution. Note: The target protein to be labeled should not be in amine-containing buffers such as Tris.
4. Add SaraFluor 650B to the protein which molar ratio should be 3:1—5:1 (SaraFluor 650B-NHS: protein). For example, add 3 µL of 10 mM SaraFluor 650B-NHS (30 nmol) to 500 µL of 3 mg/mL IgG (MW~150 kDa, 10 nmol; 3:1 in molar ratio). Mix gently with pipetting (no vortexing) and incubate for 30 minutes at 37ºC in the dark.
5. Load the dye-protein mixture to the gel filtration column and elute with elution buffer, according to the instruction of the column. SaraFluor 650B-labeled protein appears first, followed by unreacted dyes. Separate the protein from the unreacted SaraFluor 650B-NHS by fractionation.
6. Typically, the labeling ratio of the protein is about 0.9—1.8 in this condition. Add an equal volume of glycerol (final, 50%) and keep the solution at -20ºC until use. Freezing the solution below －25 ºC is not recommended.

■ Calculation of the labeling ratio

In a neutral aqueous solution, SaraFluor 650B molecule is in an equilibrium between closed form (non-fluorescent) and open form (fluorescent). For accurate measurement of the SaraFluor 650B concentration, absorbance should be measured in pH 3.5 citrate buffer (in which most of SaraFluor 650B is in the open form) as the following procedure.

First, measure the molar concentration of the protein (C_{prot}) by BCA method or by Lowry method. Next, dilute the SaraFluor 650B-labeled protein to 0.1M citrate buffer (pH 3.5) in a volume ratio of >30 (=d) and measure the absorbance at 654 nm (A_{654}). Calculate the labeling ratio using the following equation and the value shown in Table 2.

$$\text{Labeling ratio} = \frac{dA_{654}/\varepsilon}{C_{prot}}$$

■ Microscopy

It is convenient to use microscopes for PALM or STORM for the observation of SaraFluor 650B. Excitation laser power of about 30% that is usually used for Alexa Fluor® 647 observation is enough (100 W/cm^2). Optical filter set which is used for Alexa Fluor® 647 can be used (e. g. 692/40 nm bandpass emission filter, Semrock). Turn off 405 laser which is necessary for observation of Alexa Fluor® 647. SaraFluor 650B can be observed in PBS or other physiological buffer conditions. Addition of antifade reagents is recommended to reduce photobleaching.

Following the instructions of the microscope, capture hundreds to 10-thousands of images and process these images to obtain a superresolution image.

References

S.N. Uno, M. Kamiya, T. Yoshihara, K. Sugawara, K. Okabe, M.C. Tarhan, H. Fujita, T. Funatsu, Y. Okada, S. Tobita, Y. Urano (2014) *Nat. Chem.* **6**: 681-689.
DOI:10.1038/nchem.2002

F.C. Chien, C.Y. Linb, G. Abrigoa (2018) *Phys. Chem. Chem. Phys.* **20**: 27245-27255.
DOI:10.1039/C8CP02942C

Table 3. Related Products

Catalog no.	Product name	Major applications
A201-01	HaloTag® SaraFluor 650B Ligand	For superresolution imaging of HaloTag®-labeled proteins.
A202-01	SaraFluor 650B goat anti-mouse IgG	Superresolution microscopy by immunocytochemistry.
A203-01	SaraFluor 650B goat anti-rat IgG	Superresolution microscopy by immunocytochemistry.
A204-01	SaraFluor 650B goat anti-rabbit IgG	Superresolution microscopy by immunocytochemistry.
A209-01	SaraFluor 650B-maleimide	Superresolution imaging probes which can label thiols. In proteins, it can label cysteine residues.
A308-01	HaloTag® SaraFluor 650T Ligand	For STED imaging of HaloTag®-labeled proteins.