GORYO CHEMICAL

For research use only

Updated: September 25, 2019

SaraFluor™ 488B-NHS

Table 1. Product information

CAT. No.	Product name	Size	Storage upon receipt	Stability
A218-01	SaraFluor 488B-NHS	100 µg	≤−20°C, keep desiccated and protected from light.	1 year (when unopened and stored as described.)

1. About SaraFluor 488B-NHS

SaraFluor 488B (also known as HEtetTFER) is a green fluorescent probe developed exclusively for super-resolution imaging. Spontaneous blinking of this fluorophore is suitable for SMLM (single molecule localization microscopy) super-resolution imaging. This ability allows to perform the super-resolution imaging using STORM, PALM and other similar microscopy under physiological solution conditions without addition of thiols, deoxidizing reagents or exposure to strong laser irradiation to induce blinking as with general fluorescent probes. Since this product is an NHS compound that forms a covalent bond with amino group only by mixing, it can be easily used to label proteins and nucleic acids. About 5.5 - 11 nmol of proteins (or about 0.8 - 1.6 mg of IgG antibodies) can be labeled with this product.

Because the blinking frequency of this fluorescent probe is relatively low compared to that of SaraFluor 650B (also known as HMSiR), it is recommended to use higher labeling ratio (3-5) when labeling antibodies.

Table 2. Specifications

λ_{ex} (nm)*1	λ_{em} (nm)*2	ε ($M^{-1}cm^{-1}$)*3
507	530	80,000

*1 The wavelength of maximal excitation
*2 The wavelength of maximal emission
*3 Molar absorption coefficient at excitation maximum wavelength

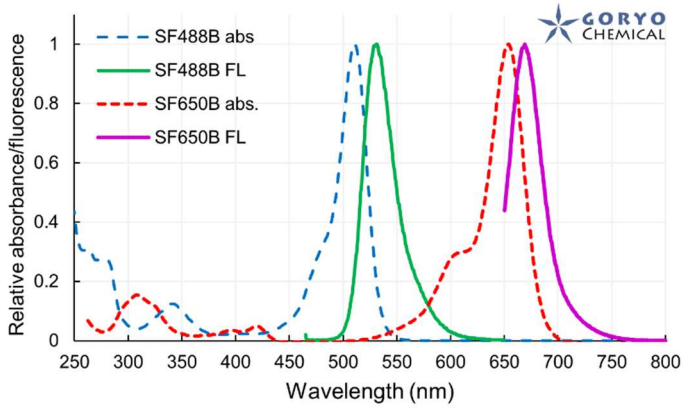
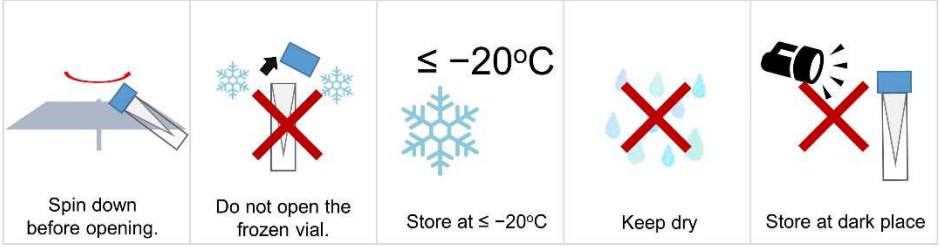


Figure 1. Fluorescence spectra

■ Storage

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



2. An example of protein labeling

■ Materials required but not provided

1. Dimethylsulfoxide, anhydrous (DMSO).
2. 0.1 M Sodium hydrogen carbonate ($NaHCO_3$, pH 8.4).
3. Phosphate buffered saline (PBS, pH 7.4) or the buffer solution suitable for target proteins.
4. A centrifugal ultrafiltration filter for protein concentration (eg: Pall Nanosep or Amicon Ultra; select one with MWCO of 1/3 - 1/6 of molecular weight of target proteins.).
5. A gel filtration column (eg: GE Healthcare NAP-5, NAP-10, NAP-25, etc.; size of the column should match the amount and molecular weight of target proteins).
6. A blocking solution (10 mg/mL BSA solution dissolved in PBS pH 7.4)

■ Preparation of reagent

1. SaraFluor 488B is yellowish red solid. Before opening the vial, spin down the solid to the bottom by a microcentrifuge or by a desktop centrifuge to collect the solid.
2. Warm the vial to the room temperature and add 11 µl of DMSO to one vial to make 10 mM stock solution. Dissolve the solid completely by careful pipetting. Solution will be also yellowish red liquid.
3. This product decomposes when exposed to moisture; its reactivity will be reduced. To prevent moisture absorption, open the product only after warming the vial to the room temperature and dissolve it directly before use.

■ IgG antibody labeling example

Labeling preparation

1. Put the blocking solution into the centrifugal ultrafiltration filter and incubate it at the room temperature for 15 minutes. Remove the blocking solution and rinse the filter thoroughly with PBS 10 times or more. Next, put 200 µL of PBS to the filter and centrifuge it to wash the filter.
2. Remove preservation solution from the gel filtration column and equilibrate it thoroughly according to the column manual.
3. Check the composition of the antibody solution you wish to label. If the solution contains proteins that are not sufficiently purified, BSA stabilizer or other proteins mixed in, the solution requires purification.

   Affinity purification methods using Protein A or Protein G are often used for antibodies. If proteins are dissolved in Tris buffer or other solution containing primary amines, solution exchange is necessary. Exchange it with a buffer solution that does not contain primary amines using a spin column, NAP-5 or other gel filtration column, or dialysis.

Antibody concentration and solution exchange

1. Measure concentration of the purified protein, put the antibody solution into the centrifugal ultrafiltration filter, and concentrate it in the centrifuge to the volume of about 1/10.
2. Concentrate the protein about 10 times in the centrifugal ultrafiltration filter and then add 0.1 M $NaHCO_3$ (pH 8.4) buffer to restore the original volume. Concentrate the solution further in the centrifuge until the antibody concentration becomes 2 mg/mL or more.

   The IgG concentration can be easily calculated from absorbance at 280 nm (A280) using Eq. 1.

$$C_{\mathrm{protein}} = \frac{A_{280} \times MW_{\mathrm{protein}}}{\varepsilon_{\mathrm{protein}}} \qquad \text{(Eq. 1)}$$

   $MW_{protein}$: Molecular weight of the protein. IgG: 150,000 g/mol

   $\varepsilon_{protein}$: Molar absorption coefficient of the protein at 280 nm. IgG: 210,000 $M^{-1}cm^{-1}$

※ Since some proteins is not stable in the 0.1 M $NaHCO_3$ (pH 8.4) buffer, consider appropriate solution conditions and act quickly after buffer replacement.

Antibody labeling

Protocols to label antibody with labeling ratio at 3-5.

1. Add the probe to the antibody solution so that the molar ratio of the probe to the protein is 10-20 and mix it well by pipetting. For example, add 4 µL (40 nmol, 20 volumes) of 10 mM SaraFluor 488 B-NHS DMSO solution to 100 µL of 3 mg/mL IgG (2 nmol at molecular weight of 150 kDa) and mix slowly by pipetting.
2. Incubate the solution overnight (for 12-18 hours) at 4°C

in a dark place. (Labeling is also often performed at the room temperature or at 37°C for 1-3 hours, but the labeling rate tends to be lower in this case.)

3. Add the reaction solution to the equilibrated gel filtration column and fractionate the protein and unreacted probe by eluting with PBS or another suitable buffer. Determine the protein concentration using the A280 measurement method for each fraction and retrieve the protein's fraction. Be careful not to include the following fraction of unbound probe.

   ※ Perform the fractionation according to the instruction manual of the gel filtration column.

Calculation of the labeling ratio

Because in a neutral solution SaraFluor 488B-NHS repeatedly changes from quenching (ring closure) to fluorescence (ring opening), complete ring opening in an acidic solution and absorbance measurement are required for accurate quantification. Calculate the labeling rate as described below.

1. To evaluate the protein concentration, measure the absorbance at 280 nm (A280) or determine the protein concentration ($C_{protein}$ in mg/mL) by the Bradford protein assay method.
2. Dilute the SaraFluor 488B labeled antibody in 0.1-1 N hydrochloric acid (10× or more buffer concentration, 0.1 N for PBS) at a ratio of 30 times or more (dilution ratio: d) and measure the absorbance at 507 nm (A_{507}). The labeling ratio can be calculated using eq. 2 if the protein concentration was determined by the Bradford method, or using eq. 3 if it was measured by A_{280}. In the latter case set CF_{280} value to 0.07.

$$\text{Labeling ratio} = \frac{A_{507} \times d \times MW_{\text{protein}}}{\varepsilon_{\text{dye}} \times C_{\text{protein}}} \quad \text{(Eq. 2)}$$

$$\text{Labeling ratio} = \frac{A_{507} \times d \times \varepsilon_{\text{protein}}}{(A_{280} - A_{507} \times d \times CF_{280}) \times \varepsilon_{dye}} \quad \text{(Eq. 3)}$$

3. Store the labeled antibody at 4°C in a dark place. It can also be stored at -20°C after adding glycerol at a final concentration of 50% to avoid freezing, or at -80°C frozen with liquid nitrogen after adding sucrose at a final concentration of 10%.

■ Fluorescence observation

SaraFluor 488B can be conveniently observed with a microscope compatible with STORM or PALM super-resolution methods. The probe can also be observed with other optical systems capable of single molecule imaging. Use the antibody concentration of 5-20 μg/mL when performing cell staining. A 488 nm laser can be used as the excitation light source. Use a light source with the light intensity of about 250W/cm^2. Use fluorescent filters for Alexa Fluor® 488, ATTO 488, FITC etc. Irradiation by 405 nm laser required for general fluorescent molecular switching is not necessary. If using a microscope for STORM or PALM methods, obtain hundreds to tens of thousands of images according to instruction manual, and create super resolution images by image processing. The image processing is supported by ImageJ, ThunderSTORM and other software.

Table 2. Related Products

CAT. No.	Product name	Description
A201-01	HaloTag® SaraFluor 650B ligand	For super resolution imaging of proteins with added HaloTag® (excitation by red laser)
A202-01	SaraFluor 650B goat anti-mouse IgG	Immunostaining for super-resolution imaging using mouse-derived primary antibodies (excitation by red laser)
A203-01	SaraFluor 650B Goat anti-rat IgG	Immunostaining for super-resolution imaging using rat-derived primary antibodies (excitation by red laser)
A204-01	SaraFluor 650B Goat anti-rabbit IgG	Immunostaining for super-resolution imaging using rabbit-derived primary antibodies (excitation by red laser)
A208-01	SaraFluor 650B-NHS	For labeling proteins, antibodies and other amino groups (red laser excitation)
A209-01	SaraFluor 650B-maleimide	For labeling thiols in cysteine residues in proteins using maleimide (red laser excitation)
A308-01	HaloTag® SaraFluor 650T ligand	For STED microscopy imaging of proteins with added HaloTag® (red laser excitation)