GORYO CHEMICAL

For research use only

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SaraFluor™-NHS

1. About SaraFluor-NHS

SaraFluor series is a product line of bright fluorophores, which are suitable for labeling proteins (e.g., antibodies) and other macromolecules. These products are also used for labeling or marking to evaluate physical properties. Among them, SaraFluor 650, 700, 720, which are derivatives of silicone rhodamine, are uniquely bright and photostable fluorophores in far-red to near-infrared wavelength range. Thus, they possess outstanding brightness and photostability.

In mildly alkaline solutions (pH 8.3-8.5), N-hydroxysuccinimide (NHS) esters quickly form covalent bonds with primary amines (e.g., in side chains of lysine residues or N-terminal amino groups of peptides). Therefore, SaraFluor-NHS provides simple labeling of proteins and macromolecules just by mixing.

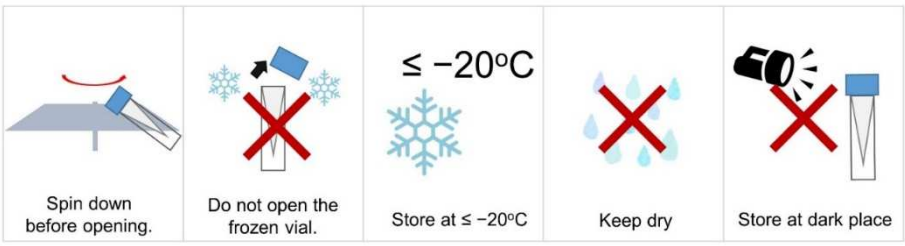
Table 1. Product information

Product name	Amount	Catalog no.
SaraFluor 488-NHS	5 nmol × 5	ST1003-10
	1 mg	ST1003-11
	1 mg × 5	ST1003-15
SaraFluor 600-NHS	5 nmol × 5	ST1006-10
	1 mg	ST1006-11
	1 mg × 5	ST1006-15
SaraFluor 650-NHS	5 nmol × 5	ST1008-10
	1 mg	ST1008-11
	1 mg × 5	ST1008-15
SaraFluor 700-NHS	5 nmol × 5	ST1010-10
	1 mg	ST1010-11
	1 mg × 5	ST1010-15
SaraFluor 720-NHS	5 nmol × 5	ST1011-10
	1 mg	ST1011-11
	1 mg × 5	ST1011-15

■ 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. A shelf-life is 1 year when it is unopened and stored as previously described. Dissolve the reagents to DMSO just before the use. We provide no warranty for the reagents which was stored as a solution.

※ 5 mg packages were previously shipped as a single 5 mg vial. New lots are shipped as 1 mg × 5 vials. For the time being, both packagings might be mixed. We appreciate your understanding.



2. Spectra and properties of SaraFluor series

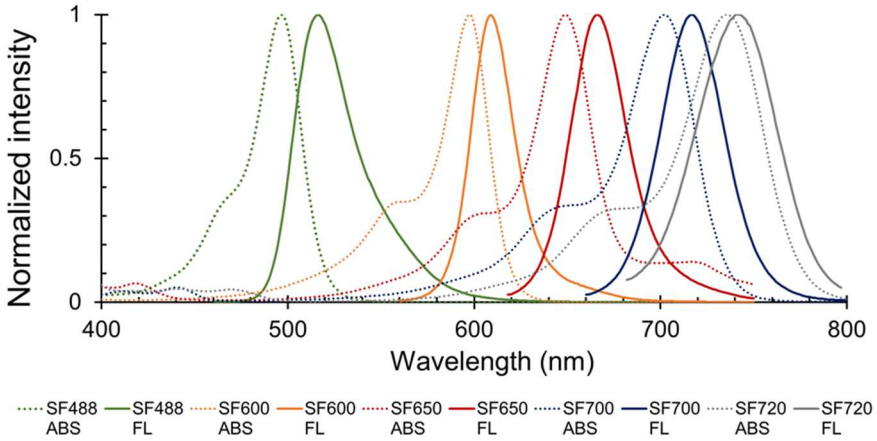


Table 2. Properties of SaraFluor-NHS

Fluorophore	Solid color/DMSO solution color	Solution volume per dye 1 mg (10 mM)	λ_{ex} (nm) *1	λ_{em} (nm) *2	ε ($M^{-1}cm^{-1}$) *3	CF_{280} *4
SaraFluor 488	Orange/Yellow	209 µL	494	514	0.82 x 10^5	0.25
SaraFluor 600	Brown/Purple	180 µL	597	607	1.3 x 10^5	0.18
SaraFluor 650	Blue/Blue	153 µL	646	660	1.1 x 10^5	0.09
SaraFluor 700	Dark blue/Light blue	148 µL	691	712	1.0 x 10^5	0.07
SaraFluor 720	Green/Light blue-green	127 µL	721	740	1.6 x 10^5	0.14

*1 Maximum excitation wavelength

*2 Maximum emission wavelength

*3 Molar extinction coefficient

*4 Correction factor for calculating labeling ratio

3. An example protocol of protein labeling

■ Materials required but not provided

- Anhydrous dimethyl sulfoxide (DMSO)
- 0.1 M sodium bicarbonate buffer ($NaHCO_3$, pH 8.4)
- An appropriate elution buffer (e.g., PBS, pH 7.4, or other buffer solutions suitable for the target protein)
- A centrifugal filter unit for concentrating proteins or desalting (e.g., Pall Nanosep or Amicon Ultra; Use the unit which has an MWCO by 1/6 to 1/3 of molecular weight.)
- A gel filtration column (e.g., NAP-5, NAP-10, NAP-25 (GE Healthcare); Please choose the column by referring to the column capacity, the amount of proteins, etc.)
- Blocking solution (e.g. 10 mg/mL BSA solution in PBS, pH 7.4)

■ Preparation of the reagent

1. Spin-down the compound by using a microcentrifuge before opening the cap. The compounds might adhere to the cap or the wall.
2. Warm the vial to the room temperature before opening the cap, to avoid moisture adsorption. Reaction with water could degrade the product and decrease its reactivity. Dissolve the powder just before use.
3. Dissolve the compound with DMSO at the volume shown in “Solution volume per dye 1 mg (10 mM)” from Table 2. Mix the solution thoroughly by pipetting. The final concentration is 10 mM. For 5 nmol vial, dissolve the solid with 5 µL of DMSO to make 1 mM stock solution.

※ Dissolve the dye with DMSO at 10-fold volume to make 1 mM stock solution if the dye dissolves poorly.

■ Protein labeling

Preparation before labeling

1. Add blocking solution to the centrifugal filter unit and leave for 10-30 min at room temperature. Then, remove the blocking solution and rinse the filter unit with elution buffer more than 5 times. After that, add elution buffer to the filter unit, centrifuge the filter unit to let the buffer flow through the filter unit.
2. Discard the preserving solution from the gel filtration column and equilibrate the column with the elution buffer according to the manufacturer's protocol.

Protein purification, concentration and buffer exchange

1. Protein purification before the labeling is required if the target protein has not been purified enough, or if amine-containing additives including protein stabilizers (e.g., BSA) has been added. Use an appropriate protein purification method. For example, an affinity purification method utilizing either protein A or protein G is commonly used for IgG purification. Additionally, buffer exchange is necessary when the solution contains primary amines (e.g., Tris buffer). Use either spin columns, gel filtration columns, or dialysis to exchange the amine-containing buffer to an appropriate buffer such as PBS.
2. Concentrate the solution by a centrifugal filter to obtain a protein of 1–3 mg/mL. Measure the absorbance at 280 nm (A_{280}) and calculate the concentration ($C_{protein}$) by using the following equation,

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

$MW_{protein}$: molecular weight of protein; 150,000 g/mol for IgG; 66,400 g/mol for BSA,

$\varepsilon_{protein}$: molar extinction coefficient of protein at 280 nm; 210,000 $M^{-1}cm^{-1}$ for IgG; 43,800 $M^{-1}cm^{-1}$ for BSA.

3. Exchange the buffer to 0.1 M $NaHCO_3$ buffer (pH 8.4) as follows. Concentrate the protein by 10-fold using a centrifugal filter unit. Next, recover the volume by adding 0.1 M $NaHCO_3$ buffer. Quickly label the protein after the buffer exchange because some proteins are not stable in the pH 8.4 buffer.

Protein labeling

1. Add the stock solution of SaraFluor-NHS to the protein solution and mix well immediately by gentle pipetting. The molar ratio of the fluorophore to the protein should be adjusted to 2–4. Typically, 2.5-fold of fluorophore gives appropriate labeling ratio. However, the molar ratio should be adjusted depending on the target protein.
2. Incubate the reaction mixture at 37°C for 0.5–1 hour in the dark. Mix the tube by tapping every 10–15 min.
3. Add the mixture to the equilibrated gel filtration column. Elute with the elution buffer to separate the labeled protein with the unconjugated free fluorophores. Measure the A_{280} of each fraction to calculate the protein concentration. Collect fractions which contain the protein. Discard fractions containing unconjugated fluorophores.
4. Concentrate on the labeled protein if necessary. Measure the absorbance and calculate a labeling ratio (r) by

$$r = \frac{A_{\lambda ex} \times \varepsilon_{\mathrm{protein}}}{(A_{280} - A_{\lambda ex} \times CF_{280}) \times \varepsilon_{ST}} \qquad \text{(Eq. 2)}$$

A_{280}, $A_{\lambda ex}$: absorbance at 280 nm and that at λ_{ex}

CF_{280}: correction factor at 280 nm (see Table 2.)

ε_{ST}: molar extinction coefficient of the fluorophore (see Table 2.)

$\varepsilon_{protein}$: molar extinction coefficient of the protein at 280 nm

■ Fluorescence observation

Observe the fluorophore using an appropriate fluorescent imager. For fluorescence microscopy of SaraFluor 488, use blue-excitation filter sets for FITC or GFP can be used. For SaraFluor 600, yellow-excitation filter sets for Texas Red, etc. can be used. Filter sets for Cy5, Cy5,5 and Cy7 can be used for SaraFluor 650, 700 and 720, respectively.