AF dyes(SE/NHS ester)

Storage: Store at -20°C protected from light.

Product Properties

Cat	Name	Ex/Em	MW	Solubility	Extinction coefficien t(ε)	DOL
BGT-CHM-05128	AF488 NHS Ester	495/519	833.93	Good soluble in DMSO, DMF, water.	76000	5-8
BGT-CHM-05129	AF555 NHS Ester	555/565	1178.49	Good soluble in DMSO, DMF, water.	150000	3-6
BGT-CHM-05130	AF647 NHS Ester	650/665	1022.02	Good soluble in DMSO, DMF, water.	270000	/

Product Introduction

AF dyes are excellent fluorescent dyes with a fluorescence emission range that covers the entire visible spectrum and beyond. AF markers exhibit brighter fluorescence and stronger photostability than other spectrally similar fluorescent dye markers. These characteristics allow you to capture images that were previously unattainable with traditional fluorescent dyes. The succinimidyl esters of AF dyes (commonly referred to as NHS esters) are available as independent reagents,providing an effective and convenient labeling solution for you to develop the best customized AF dye markers. AF dyes can selectively link to primary amines (R-NH2) located on peptides,proteins, or amine-modified nucleic acids. Unlike other reactive molecules, succinimidyl esters have very low reactivity with aromatic amines, alcohols, and phenols, including tyrosine and histidine. Succinimidyl esters are more suitable than other amine-reactive reagents (such as isothiocyanates) for linking fluorescent groups to amine-containing molecules because the amide bonds formed in the reaction are as stable as peptide bonds!

Usage Method (Taking IgG as an example)

I.**Experimental Material Preparation**

IgG: IgG should not contain amine chemicals that can react with dyes, such as amino acids, Tris, BSA, gelatin, etc. If such chemicals are present in IgG, it should be dialyzed with PBS buffer solution (pH ~7.4) in advance. The presence of azide compounds will not affect the labeling reaction.

Anhydrous DMSO

$NaHCO_3$

Dextran Gel G-25 Dialysis Column

PBS Buffer Solution (pH ~7.4)

II. Labeling Method and Steps

1) Prepare the labeled antibody: Dilute the antibody with 0.1 M $NaHCO_3$ solution (pH ~8.3) to a final concentration of 2.5mg/mL. The labeling efficiency may be higher when the protein concentration is above 5 mg/mL. Due to differences in buffer and protein purity, the more accurate labeling efficiency is determined by actual operating conditions. If the protein concentration is too low, it can be concentrated through an ultrafiltration tube. If the product is pre-diluted with phosphate buffer solution, such as PBS (without amine compounds), then about 1/10 volume of 1M $NaHCO_3$ stock solution can be directly added to the buffer to make the final concentration of $NaHCO_3$ is 0.1 M.

2) Preparation of Dye Stock Solution

Bring the AF Dye NHS Ester to room temperature, convert units, aor every 1 µmol of dye, add 100 µL of anhydrous DMSO to fully dissolve,preparing a 10 mM dye stock solution. Centrifuge briefly to settle the dye at the bottom. If a smaller amount of protein is used for the labeling reaction, the dye needs to be diluted to a lower concentration.

Note: The remaining dye storage solution should be stored at -20°C for further use. If anhydrous DMSO is used to prepare the dye storage solution, the dye can be stored for at least one month.

3) Labeling Reaction

a. Stir or vortex mix the protein solution, and gradually add 15-25 µL of dye stock solution (10 mM) to achieve a dye/protein molar ratio of 9:1 to 15:1 . The amount of dye added can be adjusted as needed to achieve the optimal DOL.

b. Stir the reaction at room temperature in the dark for 1 hour, or incubate on a shaker for 1 hour for micro-scale labeling.

4) Separation of Labeled Protein

a. Equilibrate the Sephadex G-25 gel column (10 mm × 300 mm) with PBS buffer solution (pH ~7.4).

b. Add the reaction solution to the column and elute with 1×PBS buffer solution. The first colored band to elute is the dye-protein conjugate.

Note: For small-scale labeling reactions, to avoid excessive dilution of the product, an ultrafiltration device can be used to remove free dye from the conjugate.

Ⅲ. Calculation of DOL

Determination of Protein Concentration Antibody concentration can be calculated using the following formula:

$$C(mg/mL) = \left[\frac{A280-(Amax \times Cf)}{\varepsilon 1}\right] \times \text{dilution factor}$$

C refers to the collected antibody concentration in the experiment;

Dilution factor refers to the dilution multiple during photometry measurement;

A_{280} and A_{max} are the absorbance at 280 nm and the absorbance at the absorption wavelength, respectively; **Cf** is the correction factor, refer to the product table for the AF Dye NHS Ester Cf value;

ε_1 is the extinction coefficient of the antibody, which is 1.4 for IgG.

Estimation of **DOL** is calculated using the following formula: **DOL=**

$$\left(\frac{Amax \times Mwt \times \text{dilution factor}}{\varepsilon 2 \times C}\right)$$

A_{max} is the absorbance at the absorption wavelength;
Dilution factor is the dilution multiple during photometry measurement;
Mwt refers to the molecular weight of IgG (160,000);
C is the collected antibody concentration in the experiment;**ε_2** is the molar extinction coefficient of the dye.

Ⅳ. Storage and Handling of Conjugates

For long-term storage, it is recommended to add 5-10 mg/mL of BSA and 0.01-0.03%NaN_3 solution to prevent denaturation and microbial growth. The solution should be stored in the dark at 2-8°C.

Note:

The product listed herein is for research use only and is not intended for use in human or clinical diagnosis.