Product Information

YF® Dye Succinimidyl Ester (SE)

Catalog No.	Name	Abs_{max}/Em	A_{280}/A_{max} or C_f (protein)	Extinction Coefficient(ε)	Optimal DOL (IgG)	MW
YS0033	YF®405S SE	404/431	0.7	33,000	5-10	1028
YS0019	YF®488(6)-P_4 SE	490/515	0.1	70,000	7-9	879
YS0020	YF®488(6)-X SE	490/515	0.1	70,000	7-9	745
YS0030	YF®488(5) SE	490/515	0.1	70,000	7-9	834
YS0032	YF®488(5)-P_4 SE	490/515	0.1	70,000	7-9	879
YS0036	YF®555 SE	555/565	0.08	150,000	3-6	1077
YS0027	YF®594 SE	590/617	0.56	92,000	4-7	921
YS0026	YF®594-P_4 SE	590/617	0.56	92,000	4-7	1067
YS0035	YF®647A SE	650/665	0.03	240,000	3-6	1258
YS0056	YF®750 SE	750/777	0.03	250,000	2-5	1285

Storage

Store at -20℃ and protect from light. Expiration date marked on the outer packing.

Description

YF® SE (or NHS ester) is a type of fluorescent dye with amino reactivity. The SE groups of these dyes can react with amino groups to produce stable amide bonds. Compared with other similar dyes on the market, YF® is a new generation of fluorescent dyes with stronger stability, better water solubility and better fluorescence intensity.

We also offers Super-n-Stain® Antibody Labeling Kits (S6011) in small quantities, which can label 5-100 μg antibodies in 30 minutes without the need for purification steps, which is simple and convenient.

Protocol (Take labeled IgG antibody as an example)

1. Materials

(1) IgG: The amine chemicals that can react with dyes can not be contained for IgG, such as amino acids, Tris, BSA, gelatin, etc. If IgG contains such chemicals, it should be dialyzed beforehand with PBS buffer at pH 7.4. The presence of azides does not affect the labeling reaction.

(2) Anhydrous DMSO

(3) $NaHCO_3$

(4) Dextran gel G-25 dialysis column (Note: Please use G-50 for YF640R SE dye, and other dyes can use G-25)

(5) PBS buffer solution (pH～7.4)

(6) NaN_3

(7) BSA

2. Labeled methods and steps

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(1) Prepare labeled antibodies

Dilute the antibody with a 0.1 M $NaHCO_3$ solution (pH~8.3) so that the final antibody concentration is 2.5 mg/mL. If the product is previously diluted with a phosphate buffer, such as PBS buffer (no amines), then approximately 1/10 volume of a 1M $NaHCO_3$ mother liquor can be directly added to the buffer to make the final $NaHCO_3$ concentration to 0.1 M.

Note: When the protein concentration is 2.5 mg/mL, the labeling efficiency is about 35%. The protein concentration below 2.5 mg/mL can also be used for labeling, but the labeling efficiency will decrease at this concentration. When the protein concentration is higher than 5 mg/mL, the labeling efficiency may be higher. Due to differences in buffer and protein purity, more accurate labeling efficiency is determined by actual operating conditions. If the concentration of the labeled protein is too low, it can be concentrated by ultrafiltration.

(2) Prepare Dye Storage Solution

A tube of 1 μmole of YF SE was preheated at room temperature, and 0.1 mL of anhydrous DMSO was added to the tube to prepare a dye storage solution with a concentration of 10 mM. Under appropriate conditions, vortexing can be used to fully dissolve the dye. If a smaller amount of protein is used for the labeling reaction, the dye needs to be diluted to a lower concentration.

Note: a. The remaining dye storage solution should be stored at -20℃ for subsequent use. If anhydrous DMSO is used to formulate the dye storage solution, the dye can be stored for at least one month.

b. Dyes can also be prepared with deionized water, but since the dyes will slowly hydrolyze in water, it is best to prepare a stock solution of water before use.

(3) Labeling reaction steps

a. Stir or vortex the protein solution and gradually add 15-25 μL of dye stock solution (10 mM) dropwise to make the dye/protein molar ratio in the range of 9: 1 to 15: 1. Please refer to the table above for the DOL (number of dyes bound to each protein molecule) of YF® SE-labeled IgG antibody.

b. Stir the reaction at room temperature for 1 h, or incubate for 1 h with shaking on a shaker during microlabeling.

Note: While performing the binding reaction, proceed to step 2(4) to equilibrate the dextran gel G-25 dialysis column.

(4) Isolation of labeled proteins from the reaction solution

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

b. Add the reaction solution from step (3)b to the column and elute with 1×PBS buffer. The first eluted colored band is a dye-protein conjugate.

Note: a. For small-scale labeling reactions, to avoid over-diluting the product, an ultrafiltration device can be used to remove free dye in the conjugate.

b. When the binding reaction is completed, if the dye-protein conjugate is not separated in time, 50 μL of 1M lysine can be added to stop the reaction. In most cases, this operation is not necessary because the remaining unreacted dyes have been sufficiently hydrolyzed at the end of the reaction.

3. Determine the DOL

(1) Determination of protein concentration

The antibody concentration can be calculated by the following formula: $C\ (mg / mL) = ((A_{280}-(A_{max}\times C_f)]/1.4\}\times$dilution factor.

a. C refers to the antibody concentration collected in the experiment;

b. Dilution factor refers to the dilution factor during photometric measurement;

c. A_{280} and A_{max} refer to the absorbance at 280 nm and the absorbance at the absorption wavelength, respectively;

d. C_f is the correction factor, please refer to the table above for the C_f value of YF® SE dye;

e. 1.4 refers to the extinction coefficient of IgG (mL/mg);

Note: The protein solution eluted through the column is directly used for absorbance detection. The concentration may be too

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large, so it needs to be diluted to about 0.1 mg/mL. The dilution factor (dilution factor) needs to be estimated from the initial amount of antibody (eg 5 mg) and the total volume of protein solution eluted.

(2) DOL estimation

DOL is calculated by the following formula: DOL= (A_{max}×Mwt × dilution factor)/(ε × C)

a. A_{max}, dilution factor, C value has been specified in 3(1);

b. Mwt refers to the molecular weight of IgG (~150,000);

c. ε is the molar absorption coefficient of YF® SE, please refer to the table on the first page;

d. Please refer to the table on the first page for the DOL value of the IgG antibody labeled with YF® SE. Sometimes the DOL value fluctuates slightly, but it can also get good experimental results.

Notes

1. When the protein labeled by this product needs to be stored for a long time, it is recommended to add 5-10 mg/mL BSA and 0.01-0.03% NaN_3 to prevent protein denaturation and microbial growth. Store at 4℃ in the dark. If glycerin is added to a final concentration of 50%, it can be stored at -20℃. Stable for more than one year.

2. Avoid light during operation. Stir speed should be appropriate to avoid air bubbles.

3. When installing the chromatography column, try to make the column uniform, the surface of the column is flat, and there are no bubbles or cracks.

4. Note that when loading the sample, add the sample when the column top buffer is tangent to the gel plane, and add the eluent when the sample reaches the tangent to the gel plane.

5. Other factors affecting labeling efficiency include: temperature, reaction time, pH, amount of fluorescent dye and protein, etc., which need to be carefully controlled.

6. For your safety and health, please wear lab coats and disposable gloves.

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