Product Information

Sulfo-Cy5-E Maleimide

Catalog Number: YM0068

Product Size: 1 mg

Applications Scope: Fluorescent dye, Sulfhydryl labeled dye

Parameters

Appearance: Dark green solid soluble in water, DMSO or DMF

Ex/Em: 647/665 nm

Molecular Weight: 879.12

Extinction Coefficient: 250,000

Substitute: Alexa Fluor 657, TRITC, DyLight 640, etc.

Storage

Store at -20°C and protect from light. When stored as directed, product is stable for at least 12 months.

Description

The cyanine dye Sulfo-Cy5-E is a far-red fluorescent labeling dye, which is highly hydrophilic and water-soluble, and contains free, unactivated monofunctional carboxylic acid. Sulfo-Cy5-E Maleimide is a thiol reaction form of Sulfo-Cy5-E dye. Maleimide reacts with thiol groups to form thioether coupling products. The reaction can be carried out in an amine at pH = 7. In a neutral pH environment, the Maleimide group does not react with histidine or arginine.

Cyanine dyes are excellent fluorescent dyes, and their molar absorption coefficients are unmatched among fluorescent dyes. Their succinimide esters are commonly used as fatty amino-labeling reagents and are widely used for the labeling of proteins, antibodies, nucleic acids, and other biological molecules And detection. By changing the length of the methine chain, the fluorescence emission wavelength can be changed. For each additional double bond, the redshift is exactly 100 nm according to Huoffman's rule.

The water-soluble cyanine dyes Cy3 and Cy5 have become universal fluorescent markers for gene chips. In addition, the absorption of Cy5, Cy5.5, and Cy7 in the near-infrared region is very low. They are long-wavelength dyes with high fluorescence intensity and good stability. Particularly suitable for in vivo imaging of small animals in place of radioactive elements.

Protocol (Take labeled IgG antibody as an example)

1. Materials

■ Anhydrous DMSO

■ 10-100 mM phosphate (eg PBS), Tris or HEPES buffer, pH 7.0-7.5

■ Cross-linked dextran G-25

■ (Optional) Use Tris- (2-carboxyethyl) phosphine (TCEP) to reduce disulfide bonds in proteins to generate free sulfhydryl groups.

■ NaN_3

■ BSA

2. Labeled methods and steps

2.1 Prepare labeled antibodies

a) Dissolve the antibody in buffer at room temperature to a

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final concentration of 50-100 μM (7.5-15 mg / mL for IgG).

b) Optional step: If you want to release more thiol groups from the disulfide bonds in the protein, you can add about 10-fold molar excess of TCEP at this stage. Incubate the reaction solution for about 30 min. The reduction reaction and subsequent labeling reaction are preferably performed in the presence of an inert gas (N_2 or Ar) to prevent the re-formation of disulfide bonds.

2.2 Prepare Dye Storage Solution

Remove the Sulfo-Cy5-E Maleimide dye and return to room temperature. Prepare a 10 mM dye stock solution. For 1 μmol dye: Add 100 μL of anhydrous DMSO to the vial. Vortex briefly to completely dissolve the dye, and then centrifuge for a short time to concentrate the dye on the bottom of the tube.

Note: 1) If a small amount of protein is used for the labeling reaction, the dye stock solution may need to be diluted to a lower concentration to ensure the accuracy of the volume measurement.

2) The unused storage solution can be stored in a dry place at -20 °C, protected from light, for subsequent use. If anhydrous DMSO is used to prepare the solution, the dye is stable for at least one month.

3) Dye stock solution can also be prepared with dH_2O or aqueous buffer. However, because the dye will hydrolyze over time, the water-soluble storage solution needs to be ready-to-use and cannot be stored and used again.

2.3 Labeling reaction steps

a) While stirring or vortexing the protein solution, add a certain amount of dye stock solution to produce a mixed solution of dye and protein molar ratio of 10-20. For example, for 50 μM IgG, the dye should be added to a final dye concentration of 0.5-1 mM.

b) Stir the reaction at room temperature in the dark for 2 h or overnight at 4 °C.

Note: While performing the labeling reaction, proceed to step 2.4a to prepare the glucan column.

2.4 Isolation of labeled proteins from the reaction solution

a) Equilibrate a dextran column (10 mm × 300 mm) with PBS buffer (pH ~ 7.4).

b) Load the reaction solution from step 2.3b onto the column and elute the column with PBS buffer. The first band eluting from the column corresponds to the antibody conjugate.

Note: For small-scale labeling reactions, to avoid excessive dilution of the product, an ultrafiltration device can be used to remove free dye in the conjugate. 10K ultrafiltration tubes can be used for IgG proteins; proteins with different molecular weights require different ultrafiltration tubes.

3. Determine the DOL

3.1 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.

■ C refers to the antibody concentration collected in the experiment;

■ Dilution factor refers to the dilution factor during photometric measurement;

■ A_{280} and A_{max} refer to the absorbance at 280 nm and the absorbance at the absorption wavelength (~647 nm), respectively;

■ C_f is the correction factor, Sulfo-Cy5-E Maleimide has a C_f value of 0.13;

■ 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 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.

3.2 DOL estimation

DOL is calculated by the following formula: DOL = (A_{max} ×

Mwt × dilution factor) / (ε × C)

- A_{max}, dilution factor, C value has been specified in 3.1;
- Mwt refers to the molecular weight of IgG (~ 150,000);
- ε is the molar absorption coefficient of Sulfo-Cy5-E Maleimide;

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.
2. Store at 4 °C in the dark. If glycerin is added to a final concentration of 50%, it can be stored at -20 °C. Stable for more than one year.

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