Sulfo-Cy5-E Maleimide

OM750018

Product Introduction

Sulfo - Cy5 - E, a cyanine dye, serves as a far - infrared fluorescent labeling dye. It features high hydrophilicity and water solubility, and contains free, unactivated monofunctional carboxylic acid. Sulfo - Cy5 - E Maleimide is the thiol - reactive form of the Sulfo - Cy5 - E dye. Maleimide reacts with thiol groups to form thioether conjugates, and this reaction can take place in an amine solution with a pH of 7. Under neutral pH conditions, the maleimide group does not react with histidine or arginine.

Cyanine dyes are excellent fluorescent dyes, with their molar extinction coefficients being unparalleled among fluorescent dyes. Their succinimidyl esters are commonly used fatty amino - labeling reagents, widely applied in the labeling and detection of proteins, antibodies, nucleic acids, and other biological molecules. By altering the length of the polymethine chain, the fluorescence emission wavelength can be changed. According to Huoffman's rule, each additional double bond causes a red - shift of approximately 100 nm. Water - soluble cyanine dyes such as Cy3 and Cy5 have become common fluorescent markers for gene chips. Moreover, Cy5, Cy5.5, and Cy7 have extremely low absorption backgrounds in the near - infrared region. They are long - wavelength dyes with high fluorescence intensity and good stability, making them suitable for in vivo imaging of small animals to replace radioactive elements.

Application Scope

Protein labeling

Storage and Transportation Conditions

Store at - 20°C in the dark, with the expiration date indicated on the outer packaging; transport with ice packs.

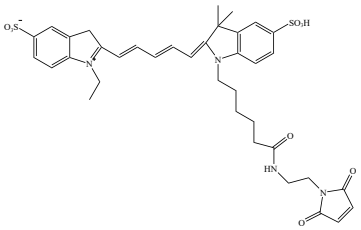
Product Features

1. **High Stability**: The product has stable performance and good labeling effects.
2. **Small Batch - to - Batch Variation**: The product is independently developed by the company, with well - controlled batch - to - batch variation.

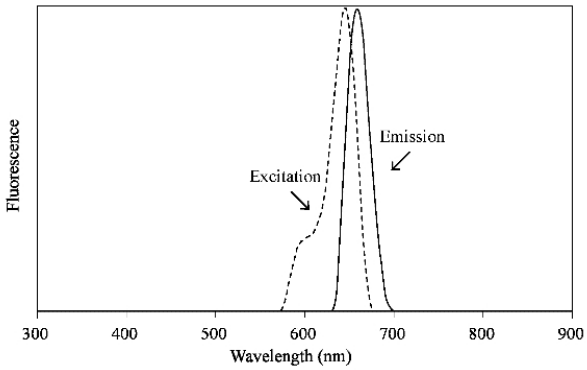
Product Parameters

- **Appearance**: A black - green solid soluble in water, DMSO, or DMF.
- **Ex/Em**: 646/661 nm.
- **Molecular Formula**: $C_{39}H_{46}N_4O_9S_2$.
- **Molecular Weight**: 778.9.
- **Extinction Coefficient**: 271,000.
- **Equivalent Efficacy**: Alexa Fluor 657, TRITC, DyLight 640, etc.

Molecular Structure Diagram:



Spectral Diagram:



Precautions

1. For long - term storage of the product, it is recommended to add BSA and NaN3 to final concentrations of 5 - 10 mg/mL and 0.01 - 0.03% respectively to prevent denaturation and microbial contamination.
2. Store the labeled antibody solution at 4°C in the dark. If glycerol with a final concentration of 50% is added, it can be stored at - 20°C. Under these conditions, the product can remain stable for one year or longer.
3. This product is solely for research purposes and must not be used for clinical diagnosis or treatment, food, or medicine. It should not be stored in ordinary residences.

4. For your safety and health, wear a lab coat and disposable gloves during operation.

Self - Prepared Materials

1. **Consumables**
    1. (1) 2 mL centrifuge tubes.
    2. (2) Ultrafiltration tubes.
2. **Reagents**
    1. (1) PBS.
    2. (2) Anhydrous DMSO.
    3. (3) Reducing agent, or TCEP, or DTT.

Operational Procedures

1. **Preparing the Antibody**
    1. Dissolve the antibody in a buffer at room temperature to a final concentration of 50 - 100 μM (IgG is 7.5 - 15 mg/mL).
    2. Optional step: If more thiol groups need to be released from the protein's disulfide bonds, add approximately 10 times the molar excess of TCEP at this stage. Incubate the reaction solution for about 30 minutes. Both the reduction reaction and the subsequent labeling reaction are preferably carried out in the presence of inert gas (N2 or Ar) to prevent the re - formation of disulfide bonds.
2. **Preparing the Dye Stock Solution**
   Take out the Sulfo - Cy5 - E Maleimide dye and bring it to room temperature. Prepare a 10 mM dye stock solution. For 1 μmol of 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 collect the dye at the bottom of the tube.
    1. Notes:
        1. (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 volume measurement.
        2. (2) Unused stock solution can be stored at - 20°C in the dark and dry for future use. If prepared with anhydrous DMSO, the dye can remain stable for at least one month.

3. (3) The dye stock solution can also be prepared in ddH2O or aqueous buffer. However, due to the hydrolysis of the dye over time, the water - soluble stock solution should be prepared fresh and not stored for later use.

3. **Labeling Reaction Steps**

1. While stirring or vortexing the protein solution, add a certain amount of the dye stock solution to achieve a dye - to - protein molar ratio of 10 - 20. For example, for 50 μM IgG, add the dye to a final concentration of 0.5 - 1 mM.
2. Stir the reaction in the dark at room temperature for 2 hours or at 4°C overnight.
3. Note: While performing the labeling reaction, carry out step 4 (1) to prepare the dextran column.

4. **Separating the Labeled Protein from the Reaction Solution**

1. Equilibrate the dextran column (10 mm × 300 mm) with PBS buffer (pH ~ 7.4).
2. Load the reaction solution from step 3 (2) onto the column and elute the column with PBS buffer. The first band eluted from the column corresponds to the antibody conjugate.
3. 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. A 10kDa ultrafiltration tube is suitable for IgG proteins; proteins with different molecular weights require different ultrafiltration tubes.

5. **Determining the DOL**

1. Determining the protein concentration
   The concentration of the dye - labeled antibody can be calculated using the following formula:
   C (mg/mL) = {[A280 - (Amax × Cf)] / 1.4} × dilution factor

(1) C refers to the concentration of the experimentally collected YF - labeled antibody.

(2) The dilution factor refers to the dilution multiple during absorbance measurement.

(3) A280 and Amax are the absorbances at 280 nm and the maximum absorption wavelength (~ 647 nm) respectively.

(4)Cf is the correction factor, and the Cf value for Sulfo - Cy5 - E Maleimide is 0.13.

Note: The protein solution eluted from the column may have too high a concentration for direct absorbance measurement, so it needs to be diluted to

approximately 0.1 mg/mL. The dilution factor (i.e., the dilution multiple) should be estimated based on the initial amount of antibody (e.g., 5 mg) and the volume of the collected eluted protein solution.

2. Estimating the DOL
   DOL is calculated using the following formula:
   DOL = (Amax × Mwt × dilution factor) / (ε × C)

(1) Amax, dilution factor, and C values are defined in 5 (1).
(2) Mwt refers to the molecular weight of IgG (~ 150,000).
(3) ε is the molar extinction coefficient of Sulfo - Cy5 - E Maleimide.