# Product Information

## Sulfo-Cy7-E, SE [Sulfonic Cyanine 7-ethyl , succinimidyl ester]

Catalog Number: C5070

Product Size: 1 mg

Application Scope: Fluorescent labeling reagent

## Parameters

Appearance: Green solid soluble in water, DMSO or DMF

Ex/ Em: 745/774 nm

Extinction Coefficient: 200,000

Molecular Weight: 818

Substitute: Alexa Fluor 750, DyLight 750 etc.

## Storage

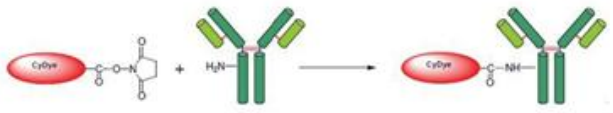
Store desiccated at -20°C and protect from light. When stored as directed, product is stable for at least 12 months.

## Description

Cyanine dye is a kind of fluorescent dye with excellent performance. Its molar absorptivity is incomparable in fluorescent dyes. Succinimide is a common fat amino labeling agent, widely used in protein, antibody, nucleic acid and other biological molecules. By changing the length of the methylene chain, the fluorescence emission wavelength can be changed. Every time a double bond is added, the red shift is about 100 nm according to the Huffman rule.

Cy3 and Cy5, the water-soluble cyanine dyes, have become the common fluorescent markers of gene chip. In addition, the absorption of Cy5, Cy5.5 and Cy7 in the near-infrared region is very low, which is a long wavelength dye with high fluorescence intensity and good stability. It is especially suitable for imaging in vivo of small living animals instead of radioactive elements.

The ratio of cyanine dye to biomolecule F/P=4～12 has better fluorescence intensity. The fluorescence probe with high F/P value will self quench and affect the biological activity of biomolecule. Monosuccinimide is commonly used to label biomolecule, but the double modified Cy Dye SE does not find cross-linking. At pH (8.5-9.4), the F/P of Cy Dye SE labeled antibody can reach 5-6 in 10 minutes, but hardly react at pH 7.0. We found that the F/P values of 1:1,5:1,10:1 and 20:1 were 0.28:1,1.16:1,2.3:1 and 4.6:1,respectively.



Labeling of cyanine dye succinimide (Cy Dye SE)

## Protocol

**1. Protein labeling with Cy dye SE (routine method)**

(1) Preparation of dye storage solution

Preheat a tube of 1 mg Cy dye SE at room temperature, add 0.122 mL of anhydrous DMSO or DMF(amine free) into the tube, and prepare a dye storage solution with a concentration of 10 mM. Under suitable conditions, the dye can be swirled to fully dissolve. 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 shall be stored at -20°C for subsequent use. If anhydrous DMSO is used to prepare dye storage solution, the dye can be kept for at least a



US EVERBRIGHT INC.
Tel:0512-88965152
Web:www.useverbright.com/

month.

(2) Calculation of dye dosage

Dosage of Cy dye SE[mg] = 8 × mass of labelled protein × molecular weight of Cy dye SE/ molecular weight of labelled protein.

Note:8,mole ratio of protein and dyes. It is an experimental empirical value, which is suitable for conventional protein and peptide labeling.

(3) Defined reaction volume

Dye labeling can be carried out in different scale, from nmole to g level. When the labeling amount is small, small volume (such as 10-20 μL) is fine, and the protein concentration should be controlled at 1-10 mg/mL.

(4) Dissolve the Cy dye SE needed for the reaction with 1/10 V DMF or anhydrous DMSO。

(5) Resuspended the protein to be labeled with 9/10 volume buffer of pH 8.3-8.5

0.1 M sodium bicarbonate solution with pH of 8.3 or a 0.1 M phosphate buffer is recommended. Pay attention to control the pH between 8.3-8.5. Avoid using buffers containing amines (sometimes Tris may can be used, but we do not recommended).

Note: when large-scale labeling (several hundred mg of SE esters) was performed, it was noted that the mixture tended to acidify over time due to the hydrolysis of SE esters. You need to monitor pH or use a stronger buffer.

(6) The dye was added to the protein solution and vortex mixed, and reacted overnight on ice or at room temperature for at least 4 hours.

(7) Purification of dye-protein conjugates with appropriate methods

Gel filtration is a commonly used method for macromolecular substances. In addition, precipitation or chromatography can also be used for separation and purification. For protein or nucleic acid purification, ethanol or acetone precipitation can also be used, too.

(8) Calculate of the concentration of dye-protein conjugate , the value of F/P

Determination of dye-protein conjugate's concentration:

$C(mg/mL) = \{[A_{280} - (A_{max} \times C_f)]/ \varepsilon\} \times$ dilution factor

C: concentration of dye-protein conjugate

Dilution factor: dilution ratio in photometry

$A_{280}$ , $A_{max}$: absorbance at 280 nm and at the maximum absorption wavelength, respectively

$C_f$ : correction factor

ε: extinction coefficient of protein (mL/mg)

Note: the protein solution eluted through the column may be too concentrated for absorbance detection directly, so it needs to be diluted to about 0.1 mg/mL. The dilution ratio(dilution factor) needs to be estimated from the initial number of antibodies (e.g. 5 mg) and the total volume of protein elution.

Calculation of F/P: For example, the molar absorption coefficient of Cy5 at 650 nm is 250000 $M^{-1}$ $cm^{-1}$, the molar absorption coefficient of protein used at 280 nm is 170000 $M^{-1}$ $cm^{-1}$; the absorption coefficient of Cy5 at 280 nm is 5% of 650 nm. Calculate the F/P value as follows.

$[Cy5\ dye] = (A_{650})/250000$

$[peptide] = [A_{280} - (0.05 \times A_{650})]/170000$

$F/P\ final = [dye]/[peptide] = \{0.68 \times A_{650}\}/ \{A_{280} - (0.05 \times A_{650})\}$

**2. Water soluble Cy Dye SE labeling OLIGO**

The amino terminated OLIGO can mark Cy Dye SE, but it is very difficult. Before marking, please wash off all residual ammonia because OLIGO is deprotected by ammonia. The sample was then dried in vacuum; then dissolved in 0.25 mL 0.5 M NaCl solution, desalted with Sephadex G-50, equilibrated to pH = 8.0 with 5.0 mM borate buffer, and then washed down with the above boric acid buffer. Then concentrate to the dry sample, dissolve in 0.1 M carbon buffer (pH 8.5-9.0); add 30 nmoles of OLIGO in 0.5 mL carbonate buffer to Cy Dye SE glass bottle at room temperature and



US EVERBRIGHT INC.
Tel:0512-88965152
Web:www.useverbright.com/

protect from light, and stir for 60 minutes. The reactants were purified by Sephadex G-50 or RP-HPLC and stored in dark after freeze-drying.

Note: oligonucleotides and oligoptides usually cannot be labeled because they are too small and often remain on the filter membrane or stick to the wall to form a membrane in the cryopreserved tube.

**3. In vivo imaging**

SPF grade BALB/C nude mice, 6-8 weeks old, 18-20 g, 24 hours before the experiment, eating and drinking freely. The animals were anesthetized by intraperitoneal injection of 2% Pentobarbital Sodium 300 μL (215 mg/kg). The nude mice were placed on the prone position in the recording dark box of the multispectral live imaging system of small animals. In the experiment, after diluting the Cy7 -E SE or biomolecule or drug labeled by Cy7 -E SE with DMSO, 200 μL (0.5 mg/g) was injected into the tail vein of nude mice [the appropriate dosage and time need to be optimized by the customer according to their own instruments and drug reagents and other conditions], one image of animal fluorescence emission in vivo was recorded every 5 minutes, and analyse the distribution of fluorescent drugs. The control rats were not injected with drugs and recorded at the same time. After recording, the organs of heart, liver, spleen, lung and kidney were dissected and imaged. The excitation wavelength of Cy7 -E SE is 700-770 nm and the emission wavelength is 790 nm. The scanning range of liquid crystal tunable filter is 780-950 nm, and the scanning step is 10 nm. The exposure time is 500 ms.

Different drug metabolism times are different. The fluorescence immediately distributes throughout the body as soon as injected into nude mice, and then gradually accumulates into the bladder, showing the characteristics of significant renal excretion. Generally, it takes 4 to 6 hours, or 30 minutes at the fastest. If it is Cy7 -E SE marker drug targeted at bone and other parts, some customers have reported that fluorescence imaging can still be detected by the imaging system in vivo a week later.

Observation of organ sections: the dissected organs were placed in 4% paraformaldehyde to be fixed for more than 4 hours, 0%, 20%, 30% sucrose in PBS successively sank to the bottom, 20 μm sections, poly lysine acid pickled slide patches, dried in the air, stain with DAPI. The laser is He-Ne of 633 nm.

## Notes

1. The unsealed powder should be store desiccated at -20℃ and protect from light; any dissolved Cy SE powder is better to be used immediately.
2. There are quenching problems with fluorescent dyes. Please avoid light to slow down the fluorescence quenching.
3. For your safety and health, please wear lab coats and disposable gloves.



US EVERBRIGHT INC.
Tel:0512-88965152
Web:www.useverbright.com/