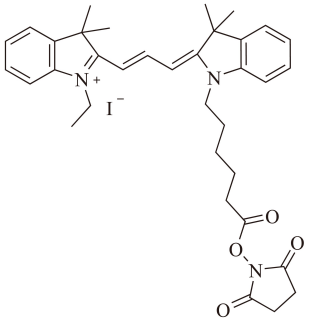
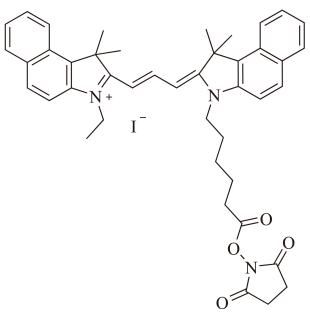
Product Information

Cy Dye SE

Product Size: 1 mg

Catalog Number	Product Name	Abs_{max}/Em (nm)	A_{280}/A_{max} or C_f(protein)	Extinction coefficient	Optimal DOL(protein)	MWt
C5077	Cy3-E SE	553/569	0.09	150,000	4-12	695.6
C5078	Cy3.5-E SE	592/610	0.22	116,000	4-12	795.8
C5045	Cy5-E SE	648/671	0.05	250,000	4-12	721.7
C5076	Cy5.5-E SE	646/662	0.03	198,000	4-12	821.8
C5083	Cy5.5-M SE	685/707	0.03	198,000	4-12	807.8
C5046	Cy7-E SE	764/788	0.029	199,000	4-12	747.7
C5060	Sulfo-Cy3-E SE	546/564	0.073	162,000	4-12	765.9
C5061	Sulfo-Cy5-E SE	645/663	0.03	250,000	4-12	792.0
C5072	Sulfo-Cy5.5-E SE	674/690	0.101	211,000	4-12	1128.4
C5070	Sulfo-Cy7-E SE	746/772	0.036	240,600	4-12	818.0
C5089	Sulfo-Cy5 bis-SE	646/662	0.03	250,000	/	975.1
C5090	Sulfo-Cy5.5 bis-SE	674/690	0.101	211,000	/	1311.6
C5091	Sulfo-Cy7 bis-SE	746/772	0.036	240,600	/	1001.2

Product Name	Molecular Formula	Molecular Structure	Color
Cy3-E SE	$C_{35}H_{42}IN_3O_4$		Red solid
Cy3.5-E SE	$C_{43}H_{46}IN_3O_4$		Purplish red solid

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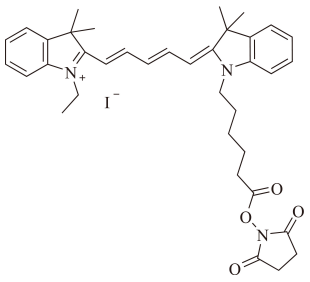
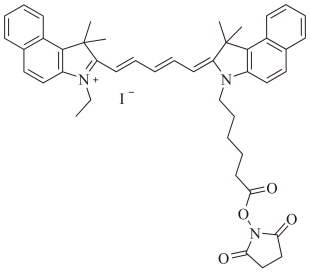
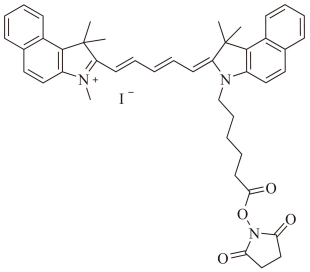
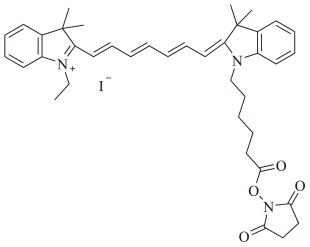
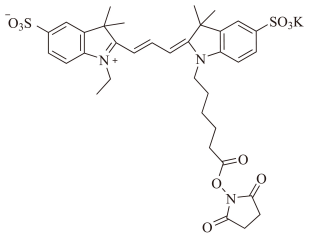
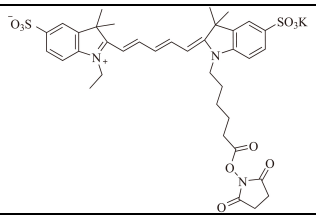
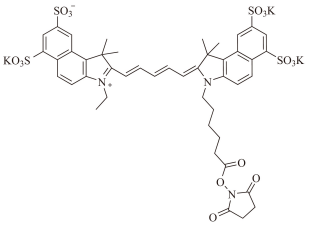
Cy5-E SE	$C_{37}H_{44}IN_3O_4$		Blue solid
Cy5.5-E SE	$C_{45}H_{48}IN_3O_4$		Dark blue solid
Cy5.5-M SE	$C_{44}H_{46}IN_3O_4$		Dark blue solid
Cy7-E SE	$C_{39}H_{46}IN_3O_4$		Green solid
Sulfo-Cy3-E SE	$C_{35}H_{40}KN_3O_{10}S_2$		Dark red solid
Sulfo-Cy5-E SE	$C_{37}H_{42}KN_3O_{10}S_2$		Dark blue solid
Sulfo-Cy5.5-E SE	$C_{45}H_{44}K_3N_3O_{16}S_4$		Dark blue solid

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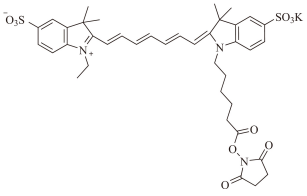
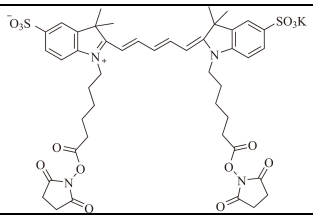
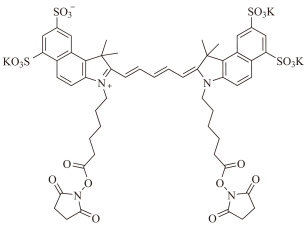
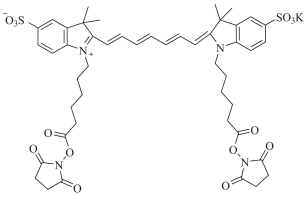
Sulfo-Cy7-E SE	$C_{39}H_{44}KN_3O_{10}S_2$		Dark green solid
Sulfo-Cy5 bis-SE	$C_{45}H_{51}KN_4O_{14}S_2$		Dark blue solid
Sulfo-Cy5.5 bis-SE	$C_{53}H_{53}K_3N_4O_{20}S_4$		Dark blue solid
Sulfo-Cy7 bis-SE	$C_{47}H_{53}KN_4O_{14}S_2$		Dark blue solid

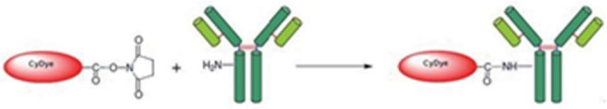
Storage

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

Description

Cy series belong to cyanine dyes, in which Sulfo-Cy SE is sulfonated highly water-soluble form, Cy SE is non-water-soluble form, both belong to monoreactive dyes, Sulfo-Cy bis-SE is sulfonated highly water-soluble form dual reactive dyes.

They are soluble in organic solvents such as DMSO, DMF, etc., and are widely used to label biomolecules such as peptides, proteins, and oligomers, especially fine proteins and easily denatured proteins. In addition to labeling biomolecules, Cy series dyes are also often used for in vivo imaging of animals. Because the spontaneous fluorescence of cells and tissues is small in the near-infrared band and the penetration depth of near-infrared light in biological tissues is large, Cy series dyes can provide higher specificity and sensitivity in the detection of complex biological systems. At the same time, Cy series dyes also have biosafety that ultraviolet dyes and isotope markers cannot have, which is conducive to monitoring the distribution of various marker molecules in live organisms.



Labeling of cyanine dye succinimide (Cy Dye SE)

Protocol

1. Protein labeling with Cy SE (routine method)

(1) Preparation of dye storage solution

Preheat a tube of 1 mg Cy SE at room temperature, add suitable amount 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

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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, it can be kept for at least a month.

(2) Calculation of dye dosage

Dosage of Cy SE [mg] = 8 × mass of labelled protein × molecular weight of Cy 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) Resuspend proteins (to be labeled) with pH 8.3-8.5 buffer

Recommend pH 8.3, 0.1 M sodium bicarbonate solution or 0.1 M phosphate buffer and the labeling effect is better when the protein concentration is controlled at 1-10 mg/mL . 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.

(4) 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.

(5) 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.

(6) Calculate of the concentration of dye-protein conjugate

Determination of dye-protein conjugate's concentration:

$$C\ (mg/mL) = \{[A_{280} - (A_{max} \times C_f)]/1.4\} \times \text{dilution factor}$$

a. C: concentration of dye-protein conjugate;

b. Dilution factor: dilution ratio in photometry;

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

c. C_f: correction factor;

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 needs to be estimated from the initial number of antibodies.

(7) Combined proportion (DOL) calculation

$$DOL = (A_{max} d M_{wt} \times \text{dilution factor})/(\varepsilon \times C)$$

a. Amax, dilution factor, C value have been clear in (6);

b. Mwt: Molecular weight of protein;

c. ε: extinction coefficient of Cy SE.

DOL value will fluctuate up and down, but good experimental results can also be obtained.

2. In vivo imaging

(1) Experimental animal preparation

Prepare the animals that need in vivo imaging according to the experimental needs. The animal grouping, negative control and positive control are set according to the specific experiment.

(2) Imaging

Cy Dye SE or Cy Dye SE labeled biomolecules or drugs were inoculated into animals by tail vein injection, subcutaneous injection, orthotopic transplantation and other methods. Select the imaging time according to the experimental requirements, perform fluorescence scanning on the whole body or local parts of the experimental animals, record the imaging pictures of fluorescence emitted from the animals, and analyze the distribution of fluorescent complexes (probes and drugs). After imaging, choose whether to dissect the viscera for imaging analysis according to the experimental needs.

Note: a. The experimental animals began fasting 6 hours before imaging to reduce the background interference caused by gastrointestinal food.

b. The optimal dosage and time need to be optimized by customers according to their own instruments, pharmaceutical reagents and other conditions.

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Notes

1. 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.

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