PC-Blue NHS Ester

Cat #: BGT-CHM-05094

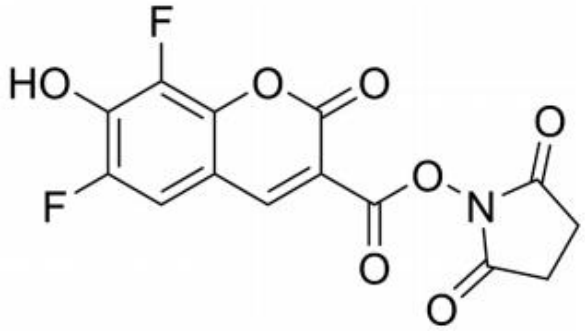
Product Introduction

PC-Blue is a bright blue fluorescent dye that can be ideally excited by a 405nm violet laser and has an excitation/emission at 410/455nm. The excitation and emission spectra of PC-Blue overlap very little with the emission spectra of green fluorescent groups, making it an ideal choice for a violet channel. The conjugates of this dye have strong fluorescence even at neutral pH. The amine-reactive PC-Blue NHS ester can be used to generate blue fluorescent bioconjugates with the maximum excitation/emission wavelengths of 410/455nm, excited by a 405nm blue diode (violet) laser. The NHS ester specifically and effectively reacts with primary amines (such as the side chains of lysine residues or aminosilane-coated surfaces) at pH 7-9, forming a stable covalent amide bond. The structure of PC-Blue NHS ester is the same as Pacific Blue™ succinimidyl ester.

Product Properties

MW: 339.21

Structural formula:



Storage

Storage conditions: Store at-20°C protected from light.

Usage Method (Taking IgG as an example)

I. Experimental Material Preparation

IgG: IgG should not contain amine chemicals that can react with the dye, such as amino acids, Tris, BSA, gelatin, etc. If such chemicals are present in IgG, it should be pre-dialyzed with a pH ~7.4 PBS buffer. The presence of azide compounds will not affect the labeling reaction.

Anhydrous DMSO

$NaHCO_3$

Sephadex G-25 gel

dialysis column.

PBS buffer solution (pH ~7.4)

II. Labeling Method and Steps

(1) Prepare the labeled antibody: Dilute the antibody with a 0.1M $NaHCO_3$ solution (pH ~8.3) to a final concentration of >2mg/mL. The labeling efficiency may be higher when the protein concentration is above 5 mg/mL. Due to differences in buffer and protein purity, the more precise labeling efficiency is determined by practical conditions. If the protein concentration is too low, it can be concentrated using an ultrafiltration tube. If the product is pre-diluted with a phosphate buffer, such as PBS buffer (free of amine compounds), you can directly add about 1/10 volume of 1M $NaHCO_3$ mother liquid in the buffer to make the final concentration of $NaHCO_3$ to 0.1 M.

(2) Prepare the dye stock solution: Preheat the PC-Blue NHS ester dye to room temperature, calculate the units, and add 100 µL of anhydrous DMSO per 1umol of dye to fully dissolve, preparing a 10mM dye stock. Perform a simple centrifugation to gather the dye at the bottom. If you are labeling with a smaller amount of protein, the dye needs to be diluted to a lower concentration.

Note: The remaining dye storage solution should be stored at -20°C for later use. If the dye storage solution is prepared with anhydrous DMSO, the dye can be stored for at least one month.

(3) Labeling reaction

a. Stir or vortex mix the protein solution and gradually add 15-25 µL of the dye storage solution (10 mM), keeping the dye/protein molar ratio between 9:1 and 15:1. The amount of dye added may need to be adjusted to achieve the optimal degree of labeling (DOL).

b. Stir the reaction at room temperature in the dark for 1 hour, or incubate on a shaker for 1 hour for micro-scale labeling.

(4) Separate the labeled protein

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

b. Add the reaction solution to the column and elute with 1×PBS buffer solution. The colored band that comes out first is the dye-protein conjugate.

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.

III. Calculate DOL

(1) Determination of protein concentration

The antibody concentration can be calculated using the following formula:

C(mg/mL)={[A280 -(Amax ×Cf)]/ε1} × dilution factor

C refers to the collected antibody concentration;

Dilution factor refers to the dilution multiple during photometry measurement;

A280 and **Amax** are the absorbance values at 280 nm and the absorption wavelength, respectively;

Cf is the correction factor, please refer to the table above for the value of Cf;

ε1 is the extinction coefficient of the antibody, and the extinction coefficient for IgG is 1.4.

(2) Estimation of DOL

DOL is calculated using the following formula: DOL = (Amax ×Mwt × dilution factor)/(ε2 ×C)

Amax is the absorbance value at the absorption wavelength;

Dilution factor is the dilution multiple during photometry measurement;

Mwt refers to the molecular weight of IgG (160,000);

C is the collected antibody concentration;

ε2 is the molar absorptivity of the dye, and the extinction coefficient for PC Blue is 30,000 $cm^{-1}M^{-1}$.

IV. Storage and Handling of Conjugates

(1) For long-term storage, it is recommended to add 5-10mg/mL of BSA and 0.01-0.03% NaN_3 solution to prevent denaturation and microbial growth. The solution should be stored in the dark at 2-8°C.

(2) The conjugate can be stored at -20°C with a final concentration of >50% glycerol.

Note:

The product listed herein is for research use only and is not intended for use in human or clinical diagnosis.