Product Data Sheet

Product Name: 6 FAM SE
Cat. No.: GC12906

Chemical Properties

Cas. No.	92557-81-8		
Chemical Name	2,5-dioxopyrrolidin-1-yl 3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-6-carboxylate		
SMILES	O=C1N(OC(C2=CC(C3(C(C=CC(O)=C4)=C4OC5=C3C=CC(O)=C5)OC6=O)=C6C=C2)=O)C(CC1)=O		
Formula	$C_{25}H_{15}NO_9$	M.Wt	473.39
Solubility	DMSO : 50 mg/mL (105.62 mM; Need ultrasonic)	Storage	Store at -20°C, protect from light
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		

Caution: Product has not been fully validated for medical applications. For research use only.
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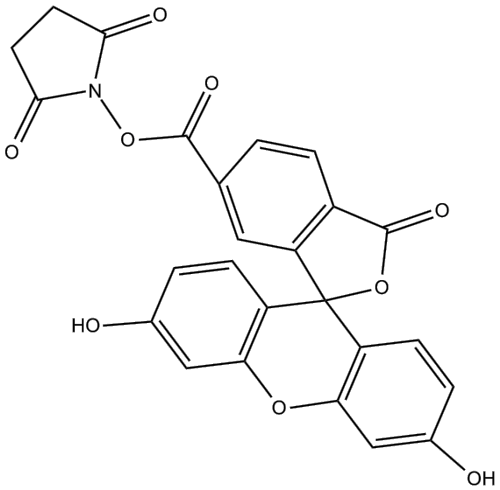
Structure



Protocol

This protocol is provided for experimental design reference only. The conditions should be optimized according to the type, molecular weight, concentration, and downstream application of the molecule to be labeled.

1. Labeling Buffer and Sample Preparation

(1) Buffer preparation: Use 0.1M sodium bicarbonate buffer (pH8.3-8.5) or 50mM borate buffer (pH8.5). Avoid buffers containing free amines, such as Tris, glycine, or ammonium salts.

(2) Sample preparation: Exchange the protein, antibody, or other primary amine-containing biomolecule into the labeling buffer. A sample concentration of 1-10mg/mL is recommended. The sample should not contain high concentrations of free amine-containing additives.

2. Preparation of the 6 FAM SE Stock Solution

Dissolve 6 FAM SE in anhydrous dimethyl sulfoxide (DMSO) to prepare a 10mM stock solution. Mix

gently and protect the solution from light. Fresh preparation immediately before use is recommended. Aliquot any unused solution and store it at -20°C protected from light, avoiding repeated freeze-thaw cycles and moisture exposure.

3. Fluorescent Labeling Reaction

(1) Calculate the required amount: Calculate the molar amount of the molecule to be labeled according to its molecular weight and concentration. A 6 FAM SE-to-target molecule molar ratio of 5:1-20:1 is recommended for preliminary optimization, with 10:1 as a suitable starting ratio.

(2) Add the dye: While gently mixing the sample, slowly add the 6 FAM SE stock solution to the reaction mixture to avoid locally high dye concentrations. The final volume fraction of DMSO in the reaction mixture should preferably be maintained below 10%.

(3) Incubate protected from light: Incubate the reaction at room temperature for 1h or at 4°C for 12-16h while protected from light. Maintain gentle mixing during the reaction and avoid vigorous agitation that may cause protein denaturation.

4. Reaction Termination and Purification

(1) Terminate the reaction: Add 1M Tris-HCl buffer (pH8.0) or 1M glycine to a final concentration of 20-50mM, followed by incubation at room temperature for 15min protected from light.

(2) Remove free dye: Remove unreacted 6 FAM SE by gel filtration, desalting-column purification, dialysis, or ultrafiltration. Exchange the labeled product into a buffer compatible with the intended downstream application.

5. Fluorescence Detection

6 FAM SE conjugates can be excited at approximately 492nm, with fluorescence emission detected at approximately 517nm. When using a fluorescence spectrophotometer, fluorescence microscope, or flow cytometer, select a green fluorescence channel compatible with this wavelength range. The exact excitation and emission maxima may vary slightly depending on the solvent, pH, and conjugated molecule.

6. Precautions

(1) The N-hydroxysuccinimide ester group of 6 FAM SE is susceptible to hydrolysis. Minimize

exposure to moisture during weighing, dissolution, and reaction, and protect the material and reaction mixture from light throughout the procedure.

(2) Excessive labeling may reduce protein activity or increase aggregation. Optimize the molar ratio, pH, and reaction time through preliminary experiments.

(3) Include an unlabeled sample control. After purification, evaluate residual free dye, protein recovery, and the activity of the labeled product.

(4) Wear a laboratory coat, gloves, and protective eyewear, and dispose of dye-containing waste according to applicable laboratory procedures.

Background

6 FAM SE is the single-isomer 6-carboxyfluorescein N-hydroxysuccinimide ester, an amine-reactive green fluorescent labeling reagent[1]. 6 FAM SE reacts with primary aliphatic amines through its N-hydroxysuccinimide ester group to form stable carboxamide-linked fluorescent conjugates that are more resistant to hydrolysis than fluorescein isothiocyanate-derived conjugates[1]. 6 FAM SE is mainly used in gene sequencing, nucleotide labeling, biomolecule conjugation, cellular uptake, and fluorescence-tracing research[1-3].

References:
[1] Adamczyk M, Fishpaugh JR, Heuser KJ. Preparation of succinimidyl and pentafluorophenyl active esters of 5- and 6-carboxyfluorescein. Bioconjug Chem. 1997;8(2):253-255. doi:10.1021/bc9600877.
[2] Zhao J, Huang X, Liu P, Qiu M, Li B, Wen Y, et al. Engineering alendronate-composed iron nanochelator for efficient peritoneal carcinomatosis treatment. Adv Sci (Weinh). 2022;9(33):e2203031. doi:10.1002/advs.202203031.
[3] Kang WS, Nguyen K, McKenna CE, Jung DH. Measurement of ototoxicity following intracochlear bisphosphonate delivery. Otol Neurotol. 2016;37(6):621-626. doi:10.1097/MAO.0000000000001042.