

Product Data Sheet

Product Name: 5(6)-FAM SE

Cat. No.: GC33461

Chemical Properties

Cas. No. 117548-22-8

SMILES OC1=CC=C2C(OC(C(C=C3)=C2C(C=CC(C(ON(C(CC4)=O)C4=O)=O)=C5)=C5C(O)=O)=CC3=O)=C1.OC6=CC=C7C(OC(C(C=C8)=C7C(C=C(C(ON(C(CC9)=O)C9=O)=O)C=C%10)=C%10C(O)=O)=CC8=O)=C6

Formula C₂₅H₁₅NO₉ M.Wt 473.39

Solubility 83.33 mg/mL in DMSO (ultrasonic and warming and heat to 50°C) Storage Store at -20°C, protect from light, stored under nitrogen

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.

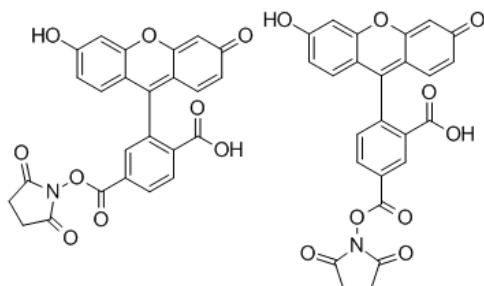
Caution: Product has not been fully validated for medical applications. For research use only.

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Structure



Protocol

Labeling protein with 5(6)-FAM SE^[1]

This protocol is adapted from research data and provided for reference only. Adjustments may be necessary based on specific experimental requirements.

1.Reagent preparation: Prepare 1M sodium phosphate buffer (pH=7.0): mix 125mL 1M NaH₂PO₄ with 875mL 1M Na₂HPO₄; prepare reaction buffer (0.1M sodium phosphate pH=7.0, 75mM KOAc pH=7.4, 2mM DTT); prepare enzyme storage buffer (20mM HEPES-KOH pH=7.4, 100mM KOAc pH=7.4, 2mM DTT, 10% glycerol); dissolve 5(6)-FAM SE (MW 473.0) in DMSO to make a 20mM stock solution, store at -20°C in dry and dark conditions.

2.Protein preparation: Dissolve the protein to be labeled in reaction buffer to a final concentration ≥2.0mg/mL. The protein should be free of other contaminating proteins and compounds containing primary amines (such as Tris, glycine, ammonium salts).

3.Labeling reaction: Calculate the moles of protein, transfer to a 1.5mL polypropylene centrifuge tube,

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add 5(6)-FAM SE (20mM DMSO stock) at a 20:1 molar ratio. Protect from light, rotate slowly at room temperature for 2-3h or at 4°C overnight. After the reaction, stop the reaction by diluting 5-10 fold with reaction buffer.

4.Purification and detection: Remove unconjugated free dye by dialysis (against 2L enzyme storage buffer at 4°C for 3h, then change to fresh buffer and continue dialysis overnight) or by gel filtration/desalting column. Then repeatedly exchange with enzyme storage buffer 6-7 times using a protein concentrator (appropriate MWCO) until no free fluorescence is observed in the flow-through, and finally concentrate the labeled protein. Perform SDS-PAGE with different concentrations of labeled protein, detect fluorescent bands under 488nm excitation to confirm successful labeling and absence of free dye. Aliquot, snap-freeze in liquid nitrogen, and store at -80°C.

5.Labeling efficiency calculation: Measure the absorbance of the sample at 280nm and 495nm. Protein concentration = $A_{280} \times \text{dilution factor} / (\epsilon_{\text{protein}} \times l)$, where $\epsilon_{\text{protein}}$ is the molar extinction coefficient of the protein ($M^{-1} \cdot \text{cm}^{-1}$), and l is the path length (usually 1cm). Fluorophore concentration = $A_{495} \times \text{dilution factor} / (68000M^{-1} \cdot \text{cm}^{-1} \times l)$. Labeling efficiency = fluorophore concentration / protein concentration.

Notes:

- (1) Minimize light exposure throughout the labeling and subsequent procedures.
- (2) The reaction system must not contain Tris, glycine, ammonium salts, or other substances with primary amines, otherwise they will competitively consume NHS esters and reduce labeling efficiency.
- (3) Free fluorophore must be completely removed; otherwise, it will affect accurate determination of labeling efficiency and subsequent applications.
- (4) The labeling reaction can preferentially target α -amino groups at pH=7.0; if enhanced reactivity with ϵ -amino groups of lysine is needed, adjust pH to >8.0.

References:

[1] Nanda JS, Lorsch JR. Labeling a protein with fluorophores using NHS ester derivitization. Methods Enzymol. 2014;536:87-94.

Background

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5(6)-FAM SE is a green fluorescent labeling reagent (Ex/Em ~494/519nm). The succinimidyl ester group of 5(6)-FAM SE can specifically covalently bind to primary amino groups to form stable amide bonds, enabling fluorescent labeling of proteins, peptides, antibodies, and amino-modified oligonucleotides. 5(6)-FAM SE can reflect cell proliferation levels and cell cycle status through cell tracing. 5(6)-FAM SE can be used in flow cytometry, immunofluorescence, Western blot, fluorescence in situ hybridization (FISH), fluorescence polarization immunoassay (FPIA), and cell dynamic tracing studies^[1-4].

References:

- [1] Bian Z, Dai C, Chu F, et al. pH biosensors based on hydrogel optical fiber. Appl Opt. 2023 Nov 1;62(31):8272-8278.
- [2] Hsu YI, Mahara A, Yamaoka T. Identification of circulating cells interacted with integrin $\alpha 4 \beta 1$ ligand peptides REDV or HGGVRLY. Peptides. 2021 Feb;136:170470.
- [3] Huang CH, Hou SY, Severance S, et al. Manipulating Diastereomeric Bicyclononynes to Sensitively Determine Enzyme Activity and Facilitate Macromolecule Conjugations. ACS Omega. 2023 Nov 20;8(48):46073-46090.
- [4] Poonia M, Küster T, Bothun GD. Organic Anion Detection with Functionalized SERS Substrates via Coupled Electrokinetic Preconcentration, Analyte Capture, and Charge Transfer. ACS Appl Mater Interfaces. 2022 May 25;14(20):23964-23972.

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