

Product Data Sheet

Product Name: Cy5 NHS ester

Cat. No.: GC12907

Chemical Properties

Cas. No.

Chemical Name (2E)-1-[6-(2,5-dioxopyrrolidin-1-yl)oxy-6-oxohexyl]-3,3-dimethyl-2-[(2E,4E)-5-(1,3,3-trimethyl-5-sulfonatoindol-1-ium-2-yl)penta-2,4-dienylidene]indole-5-sulfonate

SMILES CC1(C2=C(C=CC(=C2)S(=O)(=O)[O-])[N+](=C1C=CC=CC=C3C(C4=C(N3CCCCC(=O)ON5C(=O)CCC5=O)C=CC(=C4)S(=O)(=O)[O-])(C)C)C)C

Formula C₃₆H₄₀N₃NaO₁₀S₂ M.Wt 761.84

Solubility very good in water, good in DMF and DMSO 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.

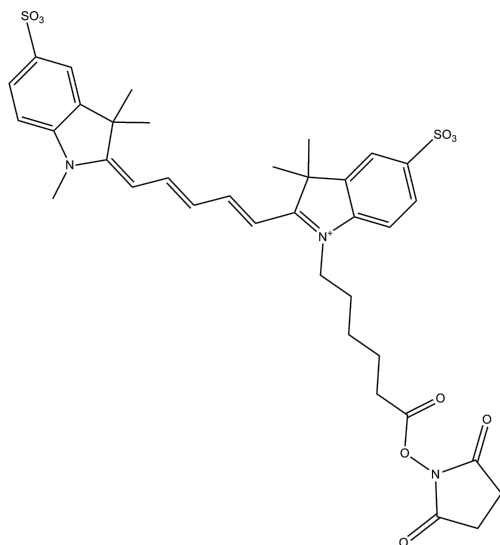
Caution: Product has not been fully validated for medical applications. For research use only.

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Structure



Protocol

Cy5 NHS ester labeling of anti-GST polyclonal antibody [1-2]

This experimental protocol is for reference only. Please adjust according to actual needs.

(1) Cy5 NHS ester preparation: Prepare Cy5 NHS ester (MW=761.84) solution at a concentration of 10mg/mL in DMSO.

(2) Calculation of dye amount: The required amount of dye is calculated based on the mass of the protein to be labeled, the molar mass of the protein and the dye, and the recommended molar excess factor. An 8-fold molar excess of NHS ester is recommended to achieve single labeling. The calculation formula is: NHS ester amount (mg) = 8 x compound mass (mg) x NHS ester molar mass (Da) / compound molar mass (Da). For example, when using Cy5 NHS ester (molar mass 761.84Da) to label 3mg anti-GST (molar mass 150kDa), the required Cy5 NHS ester mass is $8 \times 3\text{mg} \times 761.84\text{Da} / 150\text{kDa} = 0.122\text{mg}$.

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(3) Anti-GST antibody preparation: Dialyze anti-GST antibody (concentration 0.5mL at 3mg/mL) against 1L of 0.15M NaCl solution at room temperature for 4 hours. Dialyze again against fresh 1L of 0.15M NaCl solution at 4°C overnight. On the next day, dialyze against 1L of 0.1M NaHCO₃ (pH=8.3) for 4 hours. Filter the antibody solution through a 0.22µm syringe filter. Dilute a small amount of antibody with 0.1M NaHCO₃ and measure UV absorbance at 280nm to calculate the total amount of labeled antibody (IgG antibody molar extinction coefficient 170000M⁻¹cm⁻¹ at 280nm).

(4) Labeling: Add the Cy5 NHS ester solution to the antibody solution gradually in small aliquots while gently stirring. Protect the reaction from light and incubate with continuous stirring at room temperature for 45 minutes or at 4°C for 4 hours.

(5) Protein purification: Dialyze against 1L of 0.15M NaCl solution at room temperature under light protection for 4 hours to remove unlabeled Cy5 NHS ester. Dialyze again against fresh 1L of 0.15M NaCl solution at 4°C under light protection overnight. Dialyze against 1L of 0.01M PBS / 0.01% sodium azide solution at room temperature under light protection for 4 hours, then dialyze again at 4°C under light protection overnight. Filter the antibody solution through a 0.22µm syringe filter. Dilute the labeled antibody solution by an integer multiple with 0.01M PBS / 0.01% sodium azide and measure UV-Vis absorbance at 280nm (protein) and 650nm (Cy).

(6) F/P calculation: The molar extinction coefficient of Cy5 at 650nm is 250000M⁻¹cm⁻¹. The molar extinction coefficient of the protein at 280nm is 170000M⁻¹cm⁻¹. The absorption of Cy5 at 280nm is 5% of that at 650nm. Calculate the F/P value using the following formula:

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

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

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

Precautions:

(1) The stability of CyDye-labeled proteins depends on the protein itself. For example, labeled IgG can be stored at 4°C under light protection for 2 months. For longer storage, add an equal volume of glycerol and store at -20°C under light protection.

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(2) Cy5 NHS ester should be freshly prepared before use. The DMSO stock solution can be stored briefly at -20°C protected from light. Fresh preparation is recommended to ensure activity.

(3) Strictly avoid light exposure throughout the entire labeling process to prevent photobleaching of the fluorescent dye.

(4) For safety and health, please wear a lab coat and disposable gloves during operation.

References:

[1] Geng P, Li C, Quan X, et al. A thermosensor FUST1 primes heat-induced stress granule formation via biomolecular condensation in Arabidopsis. *Cell Res.* 2025 Jul;35(7):483-496.

[2] Jiang H, D'Agostino GD, Cole PA, et al. Selective protein N-terminal labeling with N-hydroxysuccinimide esters. *Methods Enzymol.* 2020;639:333-353.

Background

Cy5 NHS ester is an amine-reactive near-infrared fluorescent labeling reagent (Ex/Em=650/670nm). Cy5 NHS ester can covalently couple with primary amine groups in biomolecules such as peptides, proteins, and oligonucleotides to form stable amide bonds. Cy5 NHS ester can be used for biomolecule labeling, immunofluorescence, flow cytometry, Western blot, in vivo imaging, and nanomaterial tracking in related studies^[1-4].

References:

[1] Jeon HY, Jung SH, Jung YM, et al. Array-Based High-Throughput Analysis of Silk-Elastinlike Protein Polymer Degradation and C-Peptide Release by Proteases. *Anal Chem.* 2016 May 17;88(10):5398-405.

[2] Kim JM, Seong BL. Highly chromophoric Cy5-methionine for N-terminal fluorescent tagging of proteins in eukaryotic translation systems. *Sci Rep.* 2017;7:11642.

[3] Duan H, Zhu L, Hou J, et al. Dual-affinity peptide mediated inter-protein recognition. *Org Biomol Chem.* 2016 Dec 28;14(48):11342-11346.

[4] Zeng M, Chen X, Guan D, et al. Reconstituted Postsynaptic Density as a Molecular Platform for Understanding Synapse Formation and Plasticity. *Cell.* 2018 Aug 23;174(5):1172-1187.e16.

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