

Product Data Sheet

Product Name: Cy3.5 NHS ester (non-sulfonated)

Cat. No.: GC17971

Chemical Properties

Cas. No.

Chemical Name 2-((1E,3E)-3-(3-butyl-1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)prop-1-en-1-yl)-3-(6-(2,5-dioxopyrrolidin-1-yl)oxy)-6-oxohexyl)-1,1-dimethyl-1H-benzo[e]indol-3-ium

SMILES CC1(C)C2=C3C(C=CC=C3)=CC=C2[N+](CCCCC(ON4C(CCC4=O)=O)=O)=O)=C1/C=C/C=C5C(C)(C)C6=C(C=CC=C7)C7=CC=C6N\5CCCC

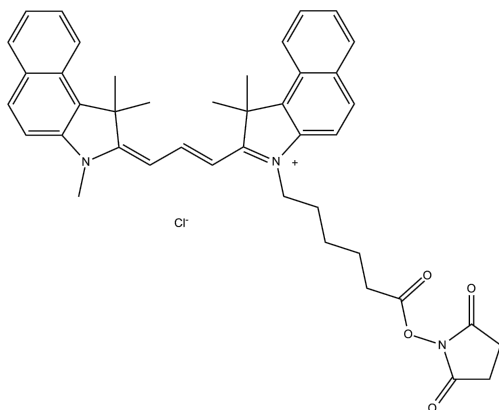
Formula $C_{42}H_{44}ClN_3O_4$ M.Wt 690.27

Solubility $\geq 69\text{mg/mL}$ in DMSO, $\geq 7.29\text{ mg/mL}$ in EtOH with ultrasonic Storage -20°C in the dark.

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.

Structure



Protocol

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

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This protocol only provides a guide, please modify it to meet your specific needs.

1. Solution preparation

(1) Stock solution: Dissolve Cy3.5 NHS ester in DMSO to prepare a stock solution with a concentration of 10mg/mL.

Note: After unused stock solution is aliquoted, store it at -20°C or -80°C in the dark to avoid repeated freezing and thawing.

(2) Working solution: Dilute the stock solution with experimental buffer to achieve the required working concentration.

Note: Please adjust the working solution concentration according to actual conditions and prepare it before use.

2. Labeling anti-GST polyclonal antibody with Cy3.5 NHS ester

If the antibodies purchased on the market contain other proteins (such as serum albumin or gelatin, etc.) or amino-containing buffers, they will affect the labeling and need to be purified before labeling.

(1) Dialyze the anti-GST antibody (concentration of 0.5mL at 3mg/mL) in 1L 0.15M NaCl solution for 4h at room temperature. Dialyze again at 4°C in the dark overnight.

(2) The next day, dialyze the solution for 4h with 1L 0.1M NaHCO₃ (pH 8.3).

(3) Filter the antibody solution with a 0.22µm syringe filter. Dilute a small amount of antibody with 0.1M NaHCO₃ and measure its UV absorbance at 280nm to calculate the total amount of labeled antibody.

(4) Prepare a Cy3.5 NHS ester solution with DMSO at a concentration of 10mg/mL. Calculate the required volume to obtain the desired ratio of Cy3.5 NHS ester to antibody (e.g. 20:1), then slowly add it to the antibody solution while stirring slowly at room temperature in the dark for 45min.

(5) Remove unlabeled Cy3.5 by dialyzing with 1L 0.15M NaCl solution at room temperature in the dark for 4h. Dialyze again at 4°C in the dark overnight.

(6) Dialyze the solution with 1L 0.01M PBS/0.01% sodium azide solution at room temperature in the

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dark for 4h, and dialyze again at 4°C in the dark overnight.

(7) Filter the antibody solution with a 0.22µm syringe filter. Dilute the labeled antibody solution with 0.01M PBS/0.01% sodium azide in multiples and measure the UV-visible absorbance at 280nm (protein) and 591nm (Cy).

(8) Freeze-dry the product into powder or store it in 0.01M PBS/0.01% sodium azide solution at -20°C in the dark.

Note: This protocol provides guidance for using Cy3.5 NHS ester for protein labeling experiments. It can be adjusted according to other literature and specific experimental requirements. The operation should be carried out in a sterile environment to prevent contaminants from interfering with the reaction. Avoid direct contact with the reaction reagents.

Background

Cy3.5 NHS ester (non-sulfonated) is a cyanine-containing fluorescent dye commonly used for labeling proteins, antibodies and small molecules, with maximum excitation/emission wavelengths of 591/604nm respectively^[1, 2]. Cy3.5 NHS ester is a high-quality active fluorescent dye optimized for amine labeling. Cy3.5 dye provides a bright orange signal^[1]. Cy3.5 NHS ester can covalently bind to the primary amine groups (such as lysine) of biological molecules (such as proteins, antibodies, and peptides) through the N-hydroxysuccinimide ester (NHS) group to form a stable amide bond, which is suitable for fluorescent labeling, live cell imaging and molecular detection^[3, 4]. The optimal molar ratio of CY dye to protein is about 10:1^[5]. The molecular weight of this product is 690.27. It is for research purposes only and is not suitable for food, drugs, medical devices or cosmetics.

References:

- [1] Chaudhuri P. AA-Dutp-Cy3 a novel fluorescent labeling agent for nucleotides[J]. Biochemical Pharmacology, 2014, 3: 1000137.
- [2] Shan H, Wang N, Gao X, et al. Fluorescent α-Conotoxin [Q1G, ΔR14] LvlB Identifies the Distribution of α7 Nicotinic Acetylcholine Receptor in the Rat Brain[J]. Marine Drugs, 2024, 22(5): 200.
- [3] Kang M S, Kong T W S, Khoo J Y X, et al. Recent developments in chemical conjugation strategies targeting native amino acids in proteins and their applications in antibody–drug conjugates[J]. Chemical Science, 2021, 12(41): 13613-13647.

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- [4] Li Y, Zhou Y, Yue X, et al. Cyanine conjugate-based biomedical imaging probes[J]. Advanced Healthcare Materials, 2020, 9(22): 2001327.
- [5] Pretzer E, Wiktorowicz J E. Saturation fluorescence labeling of proteins for proteomic analyses[J]. Analytical Biochemistry, 2008, 374(2): 250-262.

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