

Product Data Sheet

Product Name: Cy3 NHS ester (non-sulfonated)

Cat. No.: GC12618

Chemical Properties

Cas. No.

Chemical Name (2,5-dioxopyrrolidin-1-yl) 6-[(2E)-3,3-dimethyl-2-[(E)-3-(1,3,3-trimethylindol-1-ium-2-yl)prop-2-enylidene]indol-1-yl]hexanoate

SMILES CC1(C2=CC=CC=C2[N+](=C1C=CC=C3C(C4=CC=CC=C4N3CCCCC(=O)ON5C(=O)CCC5=O)(C)C)C)C

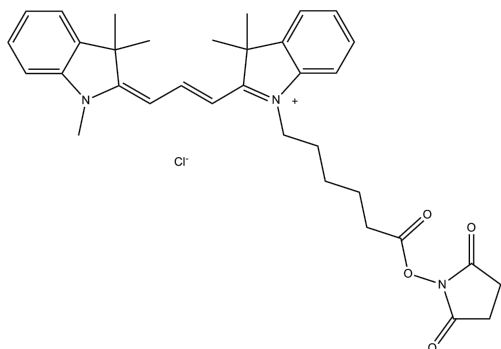
Formula $C_{34}H_{40}ClN_3O_4$ M.Wt 590.15

Solubility $\geq 59\text{mg/mL}$ in DMSO, $\geq 25.3\text{ mg/mL}$ in EtOH with ultrasonic Storage Store at -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

Protocol for Preparation and purification of Cyanine3 NHS ester –Protein (pro-FD) :

Dried dyes(Cyanine3 NHS ester) were dissolved to 1 mg/ml in anhydrous DMF and stored frozen in aliquots.

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1. Labeling reactions were performed in 20 mM NaH_2PO_4 and 300 mM NaCl (pH 6.8) buffer with 10:1 dye (Cyanine3 NHS ester) to protein molar ratio and incubated in the dark for 4 h at room temperature and then overnight at 4°C. The reactions were stopped by adding 1/10 volume 1 M Tris-HCl (pH 8) buffer, and after 30-fold dilution with ice-cold equilibration buffer, samples were applied to a 55-mm YMC-BioPro S30 column equilibrated with 20 mM NaH_2PO_4 (pH 6.8) buffer and eluted with a 0–350 mM NaCl linear gradient in the same buffer. Single-labeled Cy3 pro-FD had an A_{549}/A_{280} ratio of 4.

2. Fractions were assayed homogeneity by SDS-PAGE, and then selected fractions containing single-labeled proteins were combined and concentrated on 10 kDa cutoff concentrators to 1 mg/ml and stored frozen in aliquots.

3. The concentrations of Cy3 pro-FD was calculated based on the extinction coefficients of Cy3 ($\epsilon_{549\text{nm}} = 150,000 \text{ M}^{-1}\text{cm}^{-1}$) and molecular masses of 26.5 kDa.

This protocol only provides a guideline, and should be modified according to your specific needs.

References:

[1]. Gábor Oroszlán, Előd Kortvely, et al. MASP-1 and MASP-2 Do Not Activate Pro-Factor D in Resting Human Blood, whereas MASP-3 Is a Potential Activator: Kinetic Analysis Involving Specific MASP-1 and MASP-2 Inhibitors. *J Immunol* 15 January 2016; 196 (2): 857–865.

Background

Cyanine3 NHS ester (non-sulfonated) can be used as a reactive dye to label the amino group in a biological molecule. This reagent is commonly used to label soluble protein polypeptide oligonucleotide or DNA. Cy3 NHS ester (non-sulfonated) requires the use of an organic co-solvent (such as DMF or DMSO and acetonitrile) for the labeling^[1,2].

Cy3 (Cyanine 3) is a kind of orange fluorescence anthocyanin fluorescence dye its fluorescence is very bright, fluorescence is located in the orange region (~550nm excitation, ~570 nm emission), not sensitive to pH, and in the naked eye more sensitive visible light region, in the vast majority of fluorescence instruments can be used. Cy3 can be detected by fluorimeter imager and microscope equipped with standard TRITC filters. Cy3 in gels and solutions can be easily detected even by the naked eye due to its inherently high extinction coefficient^[3,4]

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NHS activated ester (N-hydroxysuccinimide, n-hydroxysuccinimide ester, succinimidyl ester (NHS), the most commonly used activating group in biomarker reactions, activates the carboxyl group in Cy3 molecules, allowing it to react with the amine group (main primary amine) on the target biomolecules to form a stable amide bond, thus labeling Cy3 molecules to biomolecules. Since free amine groups are common functional groups on the surface of proteins, antibodies and polypeptides (from the side chain of lysamine acid), Cy3-NHS can react directly with them. Nucleic acid molecules naturally do not contain free amine groups, and only RNA or DNA modified with amine groups can react with Cy3-NHS^[5,6].

Cy3-NHS activated ester solid can be stored at -20°C for one year, after dissolution (whether aqueous or anhydrous organic solvent) should be used as soon as possible, not long term storage.

References:

- [1]. Gao XQ, Han JX, et al. Identification of the differential expressive tumor associated genes in rectal cancers by cDNA microarray. World J Gastroenterol. 2007 Jan 21;13(3):341-8. doi: 10.3748/wjg.v13.i3.341. PMID: 17230600; PMCID: PMC4065886.
- [2]. Reid, D. A, Keegan, S, et al. Organization and dynamics of the nonhomologous end-joining machinery during DNA double-strand break repair. Proceedings of the National Academy of Sciences of the U.S.A., 2015, 112(20).
- [3]. Cho Y, et al. Mechanism of Cyanine5 to Cyanine3 Photoconversion and Its Application for High-Density Single-Particle Tracking in a Living Cell. J Am Chem Soc. 2021 Sep 8;143(35):14125-14135.
- [4]. Li H, et al. Silver enhanced ratiometric nanosensor based on two adjustable Fluorescence Resonance Energy Transfer modes for quantitative protein sensing. Biosens Bioelectron. 2017 Jan 15;87:428-432.?
- [5]. Groszewicz, et al. N-Hydroxysuccinimide-activated esters as a functionalization agent for amino cellulose: synthesis and solid-state NMR characterization. Cellulose 27, 1239–1254 (2020). <https://doi.org/10.1007/s10570-019-02864-5>
- [6]. Li M. Optimization of N-hydroxysuccinimide ester coupling with aminoallyl-modified RNA for fluorescent labeling. Bioengineered. 2020 Dec;11(1):599-606. doi: 10.1080/21655979.2020.1765487. PMID: 32449472; PMCID: PMC8291868.

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