

Product Data Sheet

Product Name: Sulfo-Cyanine5.5 NHS ester

Cat. No.: GC59219

Chemical Properties

Cas. No.

Formula $C_{44}H_{42}N_3K_3O_{16}S_4$

M.Wt 1114.37

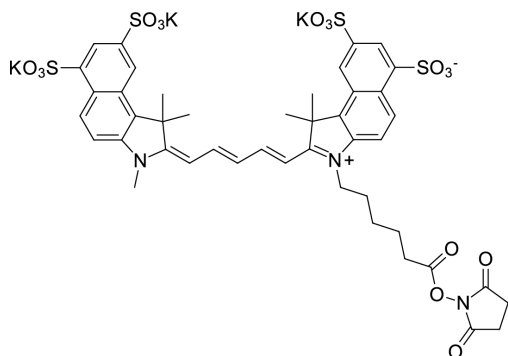
Solubility good in water, DMF, DMSO

Storage -20°C in the dark, desiccate

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

Sulfo-Cyanine5.5 NHS ester labeled anti-GST polyclonal antibody^[1-2]

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

(1) Sulfo-Cyanine5.5 NHS ester preparation: Prepare Sulfo-Cyanine5.5 NHS ester (MW=1114.37) solution at a concentration of 10mg/mL using DMSO.

(2) Calculation of dye amount: The amount of dye required for the reaction 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 coefficient. An 8-fold molar excess of NHS ester is recommended to achieve single labeling. The calculation formula is: NHS ester amount (mg) = $8 \times \text{mass of compound to be labeled (mg)} \times \text{NHS ester molar mass (Da)} / \text{molar mass of compound to be labeled (Da)}$. For example, when

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

using Cy5.5 NHS ester (molar mass 1114.37Da) to label 3mg anti-GST (molar mass 150kDa), the required Cy5.5 NHS ester mass is $8 \times 3\text{mg} \times 1114.37\text{Da}/150\text{kDa}=0.178\text{mg}$.

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

(4) Labeling: Add the Sulfo-Cyanine5.5 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 in the dark for 4 hours to remove unlabeled Sulfo-Cyanine5.5 NHS ester. Dialyze again against 1L of fresh 0.15M NaCl solution overnight at 4°C in the dark. Dialyze against 1L of 0.01M PBS/0.01% sodium azide solution at room temperature in the dark for 4 hours. Dialyze again at 4°C in the dark overnight. Filter the antibody solution using a 0.22µm syringe filter. Dilute the labeled antibody solution with an integer multiple of 0.01M PBS/0.01% sodium azide. Measure the UV-Vis absorbance at 280nm (protein) and 678nm (Cy5.5).

(6) F/P calculation: The molar extinction coefficient of Cy5.5 at 678nm is $250000\text{M}^{-1}\text{cm}^{-1}$. The molar extinction coefficient of the protein at 280nm is $170000\text{M}^{-1}\text{cm}^{-1}$. The absorption of Cy5.5 at 280nm is 18% of that at 678nm. Calculate the F/P value according to the following formulas:

$$[\text{Cy5.5 dye}] = (A_{678})/250000$$

$$[\text{peptide}] = [A_{280} - (0.18 \times A_{678})]/170000$$

$$\text{F/P}_{\text{final}} = [\text{dye}]/[\text{peptide}] = \{0.68 \times A_{678}\}/\{A_{280} - (0.18 \times A_{678})\}$$

Precautions:

(1) The stability of Cy Dye-labeled proteins depends on the protein itself. For example, labeled IgG

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

can be stored at 4°C in the dark for 2 months. For longer storage, add an equal volume of glycerol and store at -20°C in the dark.

(2) Sulfo-Cyanine5.5 NHS ester should be prepared immediately before use. DMSO stock solution can be stored briefly at -20°C in the dark, but fresh preparation is recommended to ensure activity.

(3) All steps must be performed strictly in the dark to prevent photobleaching of the fluorescent dye.

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

References:

[1] Hölte C, von Wallbrunn A, Kopka K, et al. A fluorescent photoprobe for the imaging of endothelin receptors. *Bioconjug Chem.* 2007 May-Jun;18(3):685-94.

[2] Cao G, He W, Fan Y, et al. Exploring the match between the degradation of the ECM-based composites and tissue remodeling in a full-thickness abdominal wall defect model. *Biomater Sci.* 2021 Nov 23;9(23):7895-7910.

Background

Sulfo-Cyanine5.5 NHS ester is an amine-reactive near-infrared fluorescent dye (Ex/Em=678/695nm). Sulfo-Cyanine5.5 NHS ester can react with primary amines on biomolecules such as peptides, proteins and oligonucleotides through its NHS ester group to form stable amide bonds for covalent fluorescence labeling. Sulfo-Cyanine5.5 NHS ester can be used in protein/peptide/oligonucleotide labeling, fluorescence microscopy imaging, flow cytometry and small animal in vivo tumor optical imaging related studies^[1-4].

References:

[1] Zhang W, Zhang S, Chen C, et al. The internalization mechanisms and trafficking of the pea albumin in Caco-2 cells. *Int J Biol Macromol.* 2022 Sep 30;217:111-119.

[2] Cheng Z, Levi J, Xiong Z, et al. Near-infrared fluorescent deoxyglucose analogue for tumor optical imaging in cell culture and living mice. *Bioconjug Chem.* 2006 May-Jun;17(3):662-9.

[3] Lee CM, Jeong HJ, Yun KN, et al. Optical imaging to trace near infrared fluorescent zinc oxide nanoparticles following oral exposure. *Int J Nanomedicine.* 2012;7:3203-9.

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

[4] Liu T, Wu LY, Hopkins MR, et al. A targeted low molecular weight near-infrared fluorescent probe for prostate cancer. *Bioorg Med Chem Lett*. 2010 Dec 1;20(23):7124-6.

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA