Cy5.5 acetate

Cat. No.: HY-D0924A

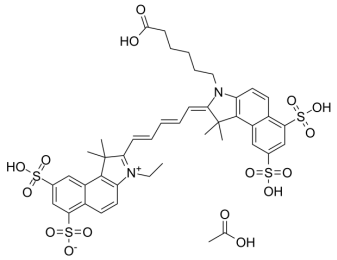
Molecular Formula: $C_{43}H_{48}N_2O_{16}S_4$

Molecular Weight: 977.11

Target: Fluorescent Dye

Pathway: Others

Storage: Please store the product under the recommended conditions in the Certificate of Analysis.



BIOLOGICAL ACTIVITY

Description

Cy5.5 acetate is a CY dye. CY, short for Cyanine, is a compound consisting of two nitrogen atoms connected by an odd number of methyl units. Cyanine compounds have the characteristics of long wavelength, adjustable absorption and emission, high extinction coefficient, good water solubility and relatively simple synthesis[1]. CY dyes are of en used for the labeling of proteins, antibodies and small molecular compounds. For the labeling of protein antibodies, the combination can be completed through a simple mixing reaction. Below, we introduce the labeling method of protein antibody labeling, which has certain reference significance[2].

In Vitro

Protocol

1.Protein Preparetion

1) In order to obtain the best labeling effect, please prepare the protein (antibody) concentration as 2 mg/mL.

2) The pH value of protein solution shall be 8.5±0.5. If the pH is lower than 8.0, 1 M sodium bicarbonate shall be used for adjustment.

3) If the protein concentration is lower than 2 mg/mL, the labeling efficiency will be greatly reduced. In order to obtain the best labeling efficiency, it is recommended that the final protein concentration range is 2-10 mg/mL.

4) The protein must be in the buffer without primary amine (such as Tris or glycine) and ammonium ion, otherwise the labeling efficiency will be affected.

2.Dye Preparation (Cy5.5)

Add anhydrous DMSO into the vial of Cy5.5 to make a 10 mM stock solution. Mix well by pipetting or vortex.

Before use, it must be activated with condensation solution (500 μg/mL) (HY-D0178) before subsequent labeling experiments can be performed.

3.Calculation of dye dosage

The amount of Cy5.5 required for reaction depends on the amount of protein to be labeled, and the optimal molar ratio of Cy5.5 to protein is about 10.

Example: assuming the required marker protein is 500 μL 2 mg/mL IgG (MW=150,000), use 100 μL DMSO dissolve 1 mg Cy5.5, the required Cy5.5 volume is 5.05 μL, and the detailed calculation process is as follows:

1) mmol (IgG) = mg/mL (IgG) ×mL (IgG) / MW (IgG) =2 mg/mL × 0.5 mL / 150,000 mg/mmol= 6.7×10-6 mmol

2) mmol (Cy5.5) = mmol (IgG) × 10 = 6.7×10-6 mmol×10 = 6.7 × 10-5 mmol

3) μL (Cy5.5) = mmol (Cy5.5) ×MW (Cy5.5) / mg/μL (Cy5.5) = 6.7 ×10-5 mmol ×753.88 mg/mmol / 0.01 mg/μL = 5.05 μL (Cy5.5)

4.Run conjugation reaction

1) A calculated volume of freshly prepared 10 mM Cy5.5 (50 μL of 500 μg/mL condensation solution can be used to activate about 10 μL dye solution) is slowly added to 0.5 mL protein sample

In solution, gently shake to mix, then centrifuge briefly to collect the sample at the bottom of the reaction tube. Don'tmix well to prevent protein samples from denaturation and inactivation.

	2) The reaction tubules were placed in a dark place and incubated gently at room temperature for 60 minutes at intervals.For 10-15 minutes, gently reverse the reaction tubules several times to fully mix the two reactants and raise the bar efficiency. 5.Purify the conjugation The following protocol is an example of dye-protein conjugate purification by using a SepHadex G-25 column. 1) Prepare SepHadex G-25 column according to the manufacture instruction. 2) Load the reaction mixture (From "Run conjugation reaction") to the top of the SepHadex G-25 column. 3) Add PBS (pH 7.2-7.4) as soon as the sample runs just below the top resin surface. 4) Add more PBS (pH 7.2-7.4) to the desired sample to complete the column purification. Combine the fractions that contain the desired dye-protein conjugate. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	Cy5.5-labeled factor VIIa is developed for imagining cancer. Cy5.5 labeled with these targeting proteins specifically localize to the tumor xenografts for at least 14 days but unconjugated Cy5.5 does not localize to any xenografts or organs. This method of imaging anti-tissue factor in the tumor VECs may be useful in detecting primary tumors and metastases as well as monitoring in vivo therapeutic responses[1]. pH/temperature sensitive magnetic nanogels conjugated with Cy5.5-labled lactoferrin (Cy5.5-Lf-MPNA nanogels) are developed as a promising contrast agent for preoperative MRI and intraoperative fluorescence imaging of glioma[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Adv Mater. 2023 Sep 5;e2306469.
- Adv Mater. 2022 Oct 4;e2207107.
- Adv Funct Mater. 2022: 2204636.
- ACS Nano. 2023 Dec 29.
- Small. 2022 Jul;18(30):e2202337.

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REFERENCES

[1]. Zhu S, et al. Visualizing cancer and response to therapy in vivo using Cy5.5-labeled factor VIIa and anti-tissue factor antibody. J Drug Target. 2015 Apr;23(3):257-65.

[2]. Ptaszek M. Rational design of fluorophores for in vivo applications. Prog Mol Biol Transl Sci. 2013;113:59-108.

[3]. Shindy, H. A. (2017). Fundamentals in the chemistry of cyanine dyes: A review. Dyes and Pigments, 145, 505–513. doi:10.1016/j.dyepig.2017.06.029

[4]. Jiang L, et al. pH/temperature sensitive magnetic nanogels conjugated with Cy5.5-labled lactoferrin for MR and fluorescence imaging of glioma in rats. Biomaterials. 2013 Oct;34(30):7418-28.

[5]. Lim B, et al. A Unique Recombinant Fluoroprobe Targeting Activated Platelets Allows In Vivo Detection of Arterial Thrombosis and Pulmonary Embolism Using a Novel Three-Dimensional Fluorescence Emission Computed Tomography (FLECT) Technology. Theranostics. 2017 Feb 26;7(5):1047-1061.

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