Cy7.5

Cat. No.: HY-D0926

CAS No.: 847180-48-7

Molecular Formula: $C_{43}H_{46}N_2O_{14}S_4$

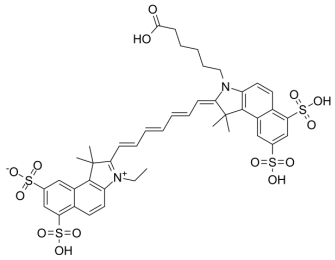
Molecular Weight: 943.09

Target: Fluorescent Dye

Pathway: Others

Storage: -20°C, protect from light

* In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



SOLVENT & SOLUBILITY

In Vitro

H_2O : 25 mg/mL (26.51 mM; ultrasonic and warming and heat to 60°C)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.0603 mL	5.3017 mL	10.6034 mL
	5 mM	0.2121 mL	1.0603 mL	2.1207 mL
	10 mM	0.1060 mL	0.5302 mL	1.0603 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.08 mg/mL (2.21 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.08 mg/mL (2.21 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Cy7.5 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 (Example for CY3-NHS ester)
Add anhydrous DMSO into the vial of CY3-NHS ester 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 CY3-NHS ester required for reaction depends on the amount of protein to be labeled, and the optimal molar ratio of CY3-NHS ester 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 CY3-NHS ester, the required CY3-NHS ester 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 (CY3-NHS ester) = mmol (IgG) × 10 = 6.7×10-6 mmol×10 = 6.7 × 10-5 mmol
3) μL (CY3-NHS ester) = mmol (CY3-NHS ester) ×MW (CY3-NHS ester) / mg/μL (CY3-NHS ester) = 6.7 ×10-5 mmol ×753.88 mg/mmol / 0.01 mg/μL = 5.05 μL (CY3-NHS ester)
4.Run conjugation reaction
1) A good volume of freshly prepared 10 mg/mL CY3-NHS ester 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.

REFERENCES

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

[2]. 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

[3]. Zhan Y, et al. In Vivo Dual-Modality Fluorescence and Magnetic Resonance Imaging-Guided Lymph Node Mapping with Good Biocompatibility Manganese Oxide Nanoparticles. Molecules. 2017 Dec 12;22(12). pii: E2208.

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

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