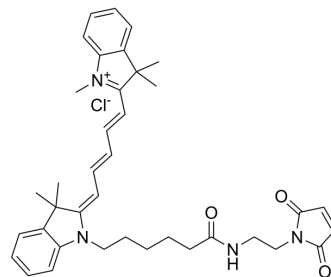


Cy5 maleimide

Cat. No.:	HY-138200
CAS No.:	1437796-65-0
Molecular Formula:	C ₃₈ H ₄₅ ClN ₄ O ₃
Molecular Weight:	641.24
Target:	DNA Stain; Fluorescent Dye
Pathway:	Cell Cycle/DNA Damage; Others
Storage:	-20°C, sealed storage, away from moisture and light * In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture and light)



SOLVENT & SOLUBILITY

In Vitro	DMSO : 125 mg/mL (194.93 mM; Need ultrasonic)				
		Solvent Concentration	Mass		
	Preparing Stock Solutions		1 mg	5 mg	10 mg
		1 mM	1.5595 mL	7.7974 mL	15.5948 mL
		5 mM	0.3119 mL	1.5595 mL	3.1190 mL
	10 mM	0.1559 mL	0.7797 mL	1.5595 mL	
Please refer to the solubility information to select the appropriate solvent.					
In Vivo	1. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.08 mg/mL (3.24 mM); Clear solution				

BIOLOGICAL ACTIVITY

Description	Cy5 maleimide 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 often 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 Preparation 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.

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 μ L 2 mg/mL IgG (MW=150,000), use 100 μ L DMSO dissolve 1 mg CY3-NHS ester, the required CY3-NHS ester volume is 5.05 μ L, and the detailed calculation process is as follows:

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

2) mmol (CY3-NHS ester) = mmol (IgG) \times 10 = 6.7×10^{-6} mmol \times 10 = 6.7×10^{-5} mmol

3) μ L (CY3-NHS ester) = mmol (CY3-NHS ester) \times MW (CY3-NHS ester) / mg/ μ L (CY3-NHS ester) = 6.7×10^{-5} mmol \times 753.88 mg/mmol / 0.01 mg/ μ L = 5.05 μ L (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't mix well to prevent protein samples from denaturation and inactivation.

2) The reaction tubes 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 tubes 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 SepHdex G-25 column.

1) Prepare SepHdex G-25 column according to the manufacture instruction.

2) Load the reaction mixture (From "Run conjugation reaction") to the top of the SepHdex 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]. Maeda K, et al. Cy5 maleimide labelling for sensitive detection of free thiols in native protein extracts: identification of seed proteins targeted by barley thioredoxin h isoforms. *Biochem J.* 2004 Mar 1;378(Pt 2):497-507.

[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

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

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