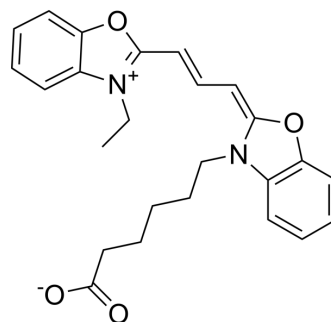


Cy2

Cat. No.:	HY-D0827
CAS No.:	260430-02-2
Molecular Formula:	C ₂₅ H ₂₆ N ₂ O ₄
Molecular Weight:	418.49
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

DMSO : ≥ 30 mg/mL (71.69 mM)
* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent		1 mg	5 mg	10 mg
	Concentration	Mass			
	1 mM		2.3895 mL	11.9477 mL	23.8954 mL
	5 mM		0.4779 mL	2.3895 mL	4.7791 mL
	10 mM		0.2390 mL	1.1948 mL	2.3895 mL

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

BIOLOGICAL ACTIVITY

Description

Cyanines are formally compounds with two nitrogen atoms linked by an odd number of methene units. The nitrogen atoms are parts of the heterocyclic units (such as indole, benzoxazol, or benzothiazol). The structures and optical properties of representative cyanine dyes used for in vivo imaging are presented^[1]. Cyanines are characterized by long wavelength, tunable absorption and emission, very high extinction coefficient (up to 300,000 M⁻¹ cm⁻¹), good water solubility, and relatively straightforward synthesis^[2].

In Vitro

- Protein Preparation
 - In order to obtain the best labeling effect, please prepare the protein (antibody) concentration as 2 mg/mL.
 - The pH value of protein solution shall be 8.5±0.5. If the pH is lower than 8.0, 1m sodium bicarbonate shall be used for adjustment.
 - 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.
 - 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.
- 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) $\text{mmol (IgG)} = \text{mg/mL (IgG)} \times \text{mL (IgG)} / \text{MW (IgG)} = 2 \text{ mg/mL} \times 0.5 \text{ mL} / 150,000 \text{ mg/mmol} = 6.7 \times 10^{-6} \text{ mmol}$
2) $\text{mmol (CY3-NHS ester)} = \text{mmol (IgG)} \times 10 = 6.7 \times 10^{-6} \text{ mmol} \times 10 = 6.7 \times 10^{-5} \text{ mmol}$
3) $\mu\text{L (CY3-NHS ester)} = \text{mmol (CY3-NHS ester)} \times \text{MW (CY3-NHS ester)} / \text{mg}/\mu\text{L (CY3-NHS ester)} = 6.7 \times 10^{-5} \text{ mmol} \times 753.88 \text{ mg/mmol} / 0.01 \text{ mg}/\mu\text{L} = 5.05 \mu\text{L (CY3-NHS ester)}$
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 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.

Attention:

1. CY dyes sensitive to light and humidity. Immediately add CY dyes solution and discard the unused part.

2. Low concentrations of sodium azide ($\leq 3 \text{ mM}$ or 0.02%) or thiomersal ($\leq 0.02 \text{ mM}$ or 0.01%) did not significantly interfere with protein labeling; However, 20-50% glycerol will reduce labeling efficiency.

3. Avoid buffering with primary amines (e.g., Tris, glycine) or ammonium ions, it competes with labeled proteins.

4. This product is only for scientific research by professionals, and shall not be used in clinical diagnosis or treatment, food or medicine.

5. For your safety and health, please wear lab coat and disposable gloves.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Altman RB, et al. Cyanine fluorophore derivatives with enhanced photostability. *Nat Methods*. 2011 Nov 13;9(1):68-71.

[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

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

Tel: 609-228-6898

Fax: 609-228-5909

E-mail: tech@MedChemExpress.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA