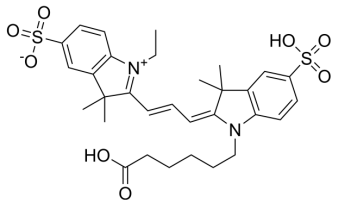
# CY3

| | |
|---|---|
| **Cat. No.:** | HY-D0822 |
| **CAS No.:** | 146368-13-0 |
| **Molecular Formula:** | $C_{31}H_{38}N_2O_8S_2$ |
| **Molecular Weight:** | 630.77 |
| **Emission (Em):** | 570 |
| **Excitation(Ex):** | 550 |
| **Target:** | Fluorescent Dye |
| **Pathway:** | Others |
| **Storage:** | -20°C, protect from light<br>* In solvent : -80°C, 6 months; -20°C, 1 month (protect from light) |



## SOLVENT & SOLUBILITY

**In Vitro**

$H_2O$ : 12.5 mg/mL (19.82 mM; ultrasonic and warming and heat to 60°C)

| Preparing Stock Solutions | Mass / Solvent / Concentration | 1 mg | 5 mg | 10 mg |
|---|---|---|---|---|
| | 1 mM | 1.5854 mL | 7.9268 mL | 15.8536 mL |
| | 5 mM | 0.3171 mL | 1.5854 mL | 3.1707 mL |
| | 10 mM | 0.1585 mL | 0.7927 mL | 1.5854 mL |

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

**In Vivo**

1. Add each solvent one by one: PBS
   Solubility: 25 mg/mL (39.63 mM); Clear solution; Need ultrasonic

## BIOLOGICAL ACTIVITY

**Description**

Cy3 (Sulfo-Cyanine3) is an orange-fluorescent label for protein and nucleic acid ($\lambda_{ex}$=554, $\lambda_{em}$=568).

**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 3.95 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= $6.7\times10^{-6}$ mmol

2) mmol (CY3-NHS ester) = mmol (IgG) × 10 = $6.7\times10^{-6}$ mmol×10 = $6.7 \times 10^{-5}$ mmol

3) μL (CY3-NHS ester) = mmol (CY3-NHS ester) ×MW (CY3-NHS ester)/mg/μL (CY3-NHS ester) = $6.7 \times10^{-5}$ mmol ×590.15 mg/mmol/0.01 mg/μL = 3.95 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.

## CUSTOMER VALIDATION

- ACS Nano. 2025 Feb 7.

See more customer validations on www.MedChemExpress.com

## REFERENCES

[1]. Lehmann B, et al. Fluorogenic "photoclick" labelling of DNA using a Cy3 dye. Org Biomol Chem. 2018 Nov 7;16(41):7579-7582.



Tel: 609-228-6898 Fax: 609-228-5909 E-mail: tech@MedChemExpress.com

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