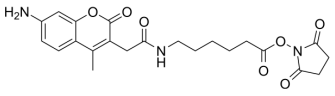
AMCA-X SE

Cat. No.:	HY-D1085
CAS No.:	216309-02-3
Molecular Formula:	$C_{22}H_{25}N_3O_7$
Molecular Weight:	443.45
Target:	Fluorescent Dye
Pathway:	Others
Storage:	-20°C, protect from light * In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



BIOLOGICAL ACTIVITY

Description

AMCA-X-SE is a coumarin derivative that generates fixed blue fluorescence and an NHS-activated ester that forms stable amide bonds with primary amine groups. It is used as a reactive dye for labeling amino groups of peptides, proteins, and oligonucleotides. Maximum excitation/emission wavelength: 354/442 nm[1].

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, 1m 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

Add DMSO into the vial of AMCA-X-SE to make a 10 mM stock solution. Mix well by pipetting or vortex.

3.Calculation of dye dosage

The amount of AMCA-X-SE required for reaction depends on the amount of protein to be labeled, and the optimal molar ratio of AMCA-X-SE to protein is about 10.

Example: assuming the required marker protein is 500 µL 2 mg/mL IgG (MW=150,000), use 100 µL DMSOdissolve 1 mg AMCA-X-SE , the required AMCA-X-SE volume is 6.63 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 (AMCA-X-SE) = mmol (IgG)×10 =6.7×10-6 mmol×10 = 6.7×10-5 mmol

3) µL (AMCA-X-SE) = mmol (AMCA-X-SE)×MW (AMCA-X-SE) / mg/µL (AMCA-X-SE) = 6.7×10-5 mmol×990.01 mg/mmol / 0.01 mg/µL = 6.63µL (AMCA-X-SE)

4.Run conjugation reaction

1) A good volume of freshly prepared 10 mg/mL AMCA-X-SE 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'tovermix 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 min at intervals.For 10-15 min, 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.

Note

1. AMCA-X-SE is sensitive to light and humidity. Immediately add AMCA-X-SE solution and discard the unused part.
2. Sodium azide (≤3 mM or 0.02%) or thiomersal (≤0.02 mM or 0.01%) with low concentrations 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 compete 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]. Hong H, et al. Surface modification of the polyethyleneimine layer on silicone oxide film via UV radiation. Applied surface science, 2009, 255(12): 6103-6106.

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