

Product Data Sheet

Product Name: Phalloidin-AMCA Conjugate

Cat. No.: GC44618

Chemical Properties

Cas. No.

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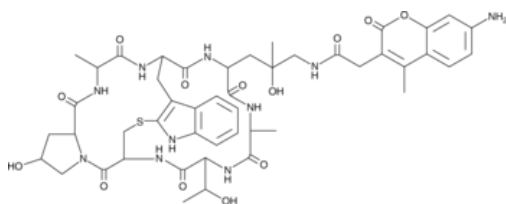
Formula $C_{47}H_{58}N_{10}O_{13}S$ M.Wt 1003.1

Solubility Soluble in DMSO Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution: ship with blue ice All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

This plan only provides a guide, please modify it to meet your specific needs.

1. Prepare dyeing solution

(1) Dye stock solution: Take the dye stock solution stored at low temperature and bring it to room temperature for at least 20 minutes. Centrifuge at low speed to concentrate the product at the bottom of the tube. Pack the stock solution according to the single dosage and store it at -20°C in the dark, stable for one year;

(2) Dye working solution: This product is provided in the form of 1000xDMSO stock solution. It is recommended to use PBS (the PBS used in this solution is 1xPBS with pH 7.4) to dilute the stock solution at a ratio of 1:1000 and dilute it to 1x dyeing work solution, pipe with a gun to mix.

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

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Notice:

- ① Please adjust and optimize the working fluid concentration according to the actual situation, and prepare it now.
- ② A PBS dilution stock solution containing 1% BSA can be used to reduce non-specific background staining and minimize the possibility of phalloidin adhering to the tube wall.

2. Cell staining

- (1) Cells are cultured on slides and grown to reach 70-80% confluence.
- (2) Aspirate the culture medium and wash the cells twice with 37°C preheated PBS.
- (3) Fix the cells with 4% paraformaldehyde dissolved in PBS and fix at room temperature for 10 minutes.

Notice:

- ① Methanol can destroy actin during the fixation process. It is recommended to use methanol-free fixative;
- ② Cells can also be fixed in PBS containing 3-4% formalin at room temperature for 10-30 minutes.
- (4) Wash cells 2-3 times with PBS at room temperature, 30 seconds each time.

Optional step ①: Treat cells with PBS containing 10 mM ethanolamine (or 0.1 M glycine) for 5 minutes to quench excess formalin;

Optional step ②: Add PBS containing 0.1% Triton X-100 to the fixed cells, let stand for 3-5 minutes to increase permeability, and then wash the cells 2-3 times with PBS.

- (5) Take about 100 μ l of the freshly prepared staining working solution to completely cover the cells on the coverslip, and incubate at room temperature in the dark for 30 minutes.

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- ① Generally, 4°C-37°C is suitable for dyeing. In order to avoid evaporation of the working solution, place the coverslip in a sealed container during the incubation process;
- ② If necessary, a nuclear staining solution with a different fluorescence spectrum than AMCA can be added at this time.
- (6) Wash cells 2-3 times with PBS at room temperature, 30 seconds each time.
- (7) Use filter paper to absorb as much liquid as possible on the cell surface, place the coverslip upside down on a glass slide with a drop of anti-fluorescence quenching agent, gently absorb the excess quenching agent with a paper towel, and then seal the coverslip with transparent nail polish All around. F-actin staining can still be maintained if the slides treated by this method are stored at 4°C in the dark for at least 6 months.
- (8) Observe the staining results under a fluorescence microscope. The maximum excitation/emission light of AMCA is 350/450nm.

Precautions:

- ① Cells can be stained in cell culture plates/copolymer dishes. Step (7) is changed to adding anti-fluorescence quenching agent dropwise into the wells to protect fluorescence;
- ② Phalloidin staining is not suitable for cells fixed with methanol or acetone. Such fixatives will destroy the structure of actin and prevent phalloidin from staining. It is recommended to use 0.2% glutaraldehyde to fix cells;
- ③ Phalloidin is sensitive to pH: If the pH value increases, the key thioether bridge in phalloidin will be cleaved, thereby losing its affinity for actin;
- ④ Phalloidin staining can be used in conjunction with antibody staining. It is recommended to add phalloidin conjugate during the incubation with primary or secondary antibodies;
- ⑤ The optimal concentration and incubation time of phalloidin conjugate depends on the specific cell type, fixation/sample preparation conditions, and/or cell/tissue permeability to the probe;
- ⑥ Suspension cells can be attached to poly-D-lysine microplates or coverslips, and then stained

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using the adherent cell protocol;

- ⑦ If the cell condition is poor, it is recommended to add serum (2-10%) to the staining solution and washing solution;
- ⑧ In some cases, a one-step method can be used for rapid phalloidin staining: 3.7% formalin and 50-100µg/mL palmitoyl lysophosphatidylcholine coupled with phalloidin at 4°C. Incubate in the conjugate for 20 minutes, then wash 3 times and mount;
- ⑨ When staining in unfixed samples, phalloidin binding reduces the rate of dissociation of actin subunits from actin filament ends, thereby stabilizing actin filaments by preventing actin filament depolymerization. ;
- ⑩ For the staining process of other sample types, in order to optimize the fixation conditions and facilitate staining, it is recommended to change the fixation time and formalin concentration within a certain range;
- ⑪ Phalloidin can be used for formalin-fixed and permeabilized tissue sections, cell cultures and other sample types as well as cell-free experiments. It can also be used for dewaxed paraffin-embedded samples, and phalloidin staining is not possible. Antigen retrieval is required;
- ⑫ The LD50 of phalloidin is 2 mg/kg. Please pay attention to protection when using it. But usually, phalloidin is used in very small amounts and does not pose a significant safety risk;
- ⑬ Fluorescent dyes all have quenching problems. Please try to avoid light to slow down fluorescence quenching;
- ⑭ For your safety and health, please wear a lab coat and disposable gloves.

Background

Phalloidin-AMCA conjugate is a blue fluorophore that specifically labels filamentous actin (F-actin). It consists of aminomethylcoumarin (AMCA; ex/em max = 350/450 nm) conjugated with phalloidin, a mycotoxin that binds F-actin. Phalloidin-AMCA conjugate is used in fixed and permeabilized tissue sections, cell cultures, or cell-free experiments.

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