Rhodamine-Phalloidin (Orange-Red)

Cat:OM750011

Product Introduction

Phalloidin is a toxin isolated from the deadly Amanita phalloides mushroom. It can specifically bind to F-actin, a double-ring peptide. Therefore, phalloidin labeled with a fluorescent dye can be very convenient for studying the distribution of F-actin. Inside phalloidin, an unusual thioether bridge is formed between cysteine and tryptophan to form an inner ring structure. When the pH increases, this thioether is cleaved, and phalloidin loses its affinity for actin.

Omnimabs® dyes are a series of new-generation fluorescent dyes developed by our company. Compared with other fluorescent dyes, they have comprehensive advantages in brightness, photostability, and water solubility. Omnimabs® fluorescent dye-labeled phalloidin can stain F-actin at the nanomolar level. In various plant or animal cells, the labeled phalloidin has a similar affinity for thick and thin filaments, with an average of one phalloidin molecule binding to each actin subunit. Different from antibodies, the binding affinity of phalloidin to actin does not vary significantly among different species. Nonspecific staining is negligible, and the contrast between the stained and unstained areas is very high. Phalloidin shifts the monomer/polymer equilibrium towards the polymerized state, reducing the polymerization critical concentration by 30 times. Phallotoxins can stabilize F-actin by inhibiting the depolymerization of cytochalasin, potassium iodide, and elevated temperatures. Because phalloidin conjugates are very small, with a diameter of approximately 12 - 15 Å and a molecular weight of less than 2000 Daltons, many actin-binding proteins, including myosin, tropomyosin, and post-troponin, can still bind to phalloidin-labeled actin. More importantly, phalloidin-labeled actin filaments remain functional. Labeled glycerinated muscle fibers still contract, and labeled actin filaments can still move. Moreover, fluorescently labeled phalloidin can also be used for quantitative studies of F-actin in cells.

Application Scope

Cytoskeleton localization staining

Product Information

Catalog Number	Name	Abs/Em (nm)	Specifications
OM750005	Omnimabs®488-Phalloidin (Green)	490/515	50T, 300T
OM750006	Omnimabs®555-Phalloidin (Orange-Red)	555/565	50T, 300T

Catalog Number	Name	Abs/Em (nm)	Specifications
OM750007	Omnimabs®594-Phalloidin (Red)	590/617	50T, 300T
OM750008	Omnimabs®633-Phalloidin (Far-Red)	630/650	50T, 300T
OM750009	Omnimabs®640-Phalloidin (Far-Red)	642/662	50T, 300T
OM750010	Omnimabs®680-Phalloidin (Near-Infrared Red)	681/698	50T, 300T
OM750011	Rhodamine-Phalloidin (Orange-Red)	546/575	50T, 300T

Storage and Transportation Conditions

Store at -20 °C in a dry and dark place. The expiration date is indicated on the outer packaging. If prepared into an aqueous solution, it should be aliquoted and stored. Ship with ice packs.

Product Features

- Complete variety: Covers from green fluorescence to near-infrared fluorescence, providing customers with many choices.
- High specificity: Specifically binds to F-actin in the cytoskeleton.
- Strong stability: The Omnimabs series is independently developed by our company, with strong fluorescence brightness and good anti-quenching properties.

Precautions

1. Before use, centrifuge the product briefly to the bottom of the tube, and then proceed with subsequent experiments.
2. This product is in the form of lyophilized powder. It is difficult to observe due to its small amount. Before use, centrifuge it briefly, dissolve it with an appropriate solvent, and the dissolved solution is almost colorless.
3. If the staining effect is not satisfactory, pre-incubate with PBS containing 1% BSA for 20 - 30 min, which can improve the staining effect.
4. This product is only for scientific research and cannot be used for clinical diagnosis or treatment, nor can it be used in food or medicine. It should not be stored in ordinary residences.

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

Self-prepared Materials

1. Reagents

(1) ddH_2O (2) Methanol (3) PBS (4) Anti-fluorescence quenching mounting medium

2. Instruments

Fluorescence microscope

Operation Steps

1. Preparation of Stock Solution

Omnimabs® dye/rhodamine-labeled phalloidin: Take an appropriate amount of methanol or sterile water to dissolve the lyophilized powder in the tube, and prepare a stock solution of 200 T/mL (for a 300 T specification dye, add 1.5 mL of liquid; for a 50 T specification dye, add 0.25 mL of liquid).

One unit (T) of Omnimabs® dye/rhodamine-labeled phalloidin is defined as the amount of dye used to stain a slide loaded with cells. For Omnimabs® dye/rhodamine-labeled phalloidin, the recommended dilution ratio for use is 1:40 - 1:200. One unit is equivalent to adding 1 - 5 μL of a 200T/mL stock solution to a total staining volume of 200 μL.

Note: The dilution ratio can be adjusted appropriately according to the actual staining effect.

2. Fixed Cell Staining

The following protocol is for the staining of adherent cells grown on glass coverslips or 8-well chamber slides. Phalloidin can also be used to stain fixed frozen tissue sections, but it is not recommended for staining paraffin tissue sections.

(1) Wash the cells 3 times with PBS.

(2) Fix the cells with PBS solution containing 4% formaldehyde at room temperature for 20 min.

Note: Methanol can damage actin during the fixation process. Therefore, it is best to avoid fixatives containing any methanol. The preferred fixative is formaldehyde without methanol.

(3) Wash the cells 3 times with PBS.

(4) Permeabilize the cells with PBS solution containing 0.4% Triton X-100 at room temperature for 10 min.

(5) Wash the cells 3 times with PBS.

(6) Dilute 1 - 5 μL of the fluorescently labeled phalloidin stock solution with 200 μL of PBS, add it to a coverslip or well, and incubate at room temperature for 20 min for staining.

Note: The staining volume can be adjusted according to the sample situation. To avoid the evaporation of the staining solution during the incubation process, the coverslip can be placed in a sealed container.

(7) Wash the cells 2 - 3 times with PBS.

(8) Observe under a fluorescence microscope. The labeled phalloidin has good photostability. The sample can be imaged in PBS, but for the best effect, an anti-fluorescence quenching agent can also be used for observation.

3. Live Cell Staining

Fluorescently labeled phalloidin is not cell-permeable and is therefore not widely used for live cell labeling. However, it has been reported that live cells may be labeled through pinocytosis or an unknown mechanism. Generally, more dye is required when staining live cells. Alternatively, fluorescently labeled phalloidin can also be injected into cells to monitor actin distribution and cell movement.

4. Experimental Effect Diagram (Figure 1)

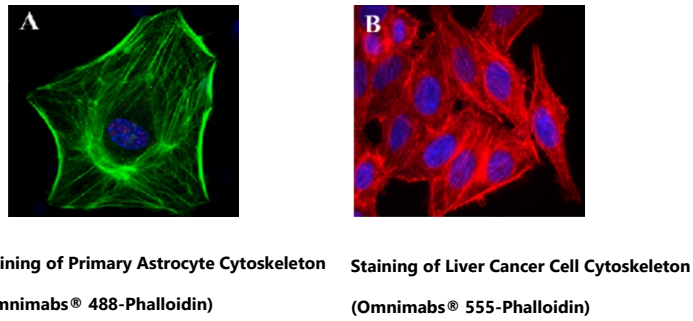


Figure 1 Staining Effect Diagram of Phalloidin

FAQ

1. Question: Why is the tube empty when I receive it?

Answer: This product is in the form of lyophilized powder. It is difficult to observe due to its small amount. Before use, centrifuge it briefly and dissolve it with an appropriate solvent.

2. Question: Can methanol be used to fix cells during the phalloidin staining of the cytoskeleton?

Answer: Methanol will damage Actin protein. Therefore, fixatives containing methanol cannot be used, and formaldehyde without methanol should be used instead.

3. Question: Can this product be used to stain paraffin sections?

Answer: No. The embedding reagents in paraffin sections can damage F-actin in actin, resulting in a situation where staining cannot be achieved.