

Phalloidin, Alexa Fluor® 488 Conjugate

Cat. No. PA-3010
Storage upon receipt: -20°C
Desiccate
Protect from light
Solvent for stock: Methanol

Instructions for Use

Phalloidin contains an unusual thioether bridge between a cysteine and tryptophan residue that forms an inner ring structure. At elevated pH, this thioether is cleaved and the toxin loses its affinity for actin. Fluorescent and biotinylated phallotoxins stain F-actin at nanomolar concentrations and are extremely water soluble, thus providing convenient probes for labeling, identifying and quantitating F-actin in tissue sections, cell cultures or cell-free experiments¹⁻³. Labeled phallotoxins have similar affinity for both large and small filaments, binding in a stoichiometric ratio of about one phalloxin molecule per actin subunit in muscle and nonmuscle cells from many different species of plants and animals.

Unlike antibodies, the binding affinity does not change appreciably with actin from different species or sources. Nonspecific staining is negligible, and the contrast between stained and unstained areas is extremely large. It has been reported that phallotoxins are unable to bind to monomeric G-actin¹. Phallotoxins shift the monomer/polymer equilibrium toward the polymer, lowering the critical concentration for polymerization up to 30-fold^{3,4}. Phallotoxins also stabilize F-actin, inhibiting depolymerization by cytochalasins, potassium iodide and elevated temperatures.

Because the phalloxin conjugates are small, with an approximate diameter of 12–15 Å and molecular weight of <2000 daltons, a variety of actin-binding proteins — including myosin, tropomyosin, troponin and DNase I — can still bind to actin after treatment with phallotoxins. Even more significantly, phalloxin-labeled actin filaments remain functional; labeled glycerinated muscle fibers still contract, and labeled actin filaments still move on solid-phase

myosin substrates^{6,7}. Fluorescent phallotoxins can also be used to quantitate the amount of F-actin in cells^{8,9}.

Materials

Upon receipt, these products should be stored frozen at -20°C, desiccated and protected from light. Once reconstituted in methanol, the stock solutions are stable for at least one year when stored frozen at -20°C, desiccated and protected from light.

Preparation of Stock Solution

Phalloidin, Alexa Fluor® 488 Conjugate is supplied as lyophilized solid in a vial containing 150 units of product. The vial contents (which are hardly visible) should be dissolved in 0.75 ml methanol to yield a final concentration of 200 units/ml, which is equivalent to approximately 6.6 µM.

One unit of phalloxin is defined as the amount of material used to stain one microscope slide of fixed cells, according to the following protocol (see step 1.6), and is equivalent to 5 µl of methanolic stock solution for the fluorescent phallotoxins.

Procedures for Staining Slides

This procedure may not be optimum for a particular experimental system, but has yielded consistent results in most instances. The following protocol describes the staining procedure for adherent cells grown on glass coverslips.

Formaldehyde-Fixed Cells

- 1.1 Wash cells twice with prewarmed phosphate-buffered saline, pH 7.4 (PBS).
- 1.2 Fix the sample in 3.7% formaldehyde solution in PBS for 10 minutes at room temperature (note A).
- 1.3 Wash two or more times with PBS.
- 1.4 Place each coverslip in a glass petri dish and extract it with a solution of acetone at -20°C or 0.1% Triton® X-100 in PBS for 3 to 5 minutes.
- 1.5 Wash two or more times with PBS.

- 1.6 When staining with the fluorescent phallotoxin, dilute 5 µl methanolic stock solution into 200 µl PBS for each coverslip to be stained. To reduce nonspecific background staining with these conjugates, add 1% bovine serum albumin (BSA) to the staining solution.
It may also be useful to pre-incubate fixed cells with PBS containing 1% BSA for 20–30 minutes prior to adding the phallotoxin staining solution. When staining more than one coverslip, adjust volumes accordingly. For a stronger signal, use 2 or 3 units per coverslip.
- 1.7 Place the staining solution on the coverslip for 20 minutes at room temperature (generally, any temperature between 4°C and 37°C is suitable). To avoid evaporation, keep the coverslips inside a covered container during the incubation.
- 1.8 Wash two or more times with PBS.
- 1.9 For long-term storage, the cells should be air dried and then mounted in a permanent mountant such as ProLong® or Cytoseal™. Specimens prepared in this manner retain actin staining for at least six months when stored in the dark at 2–6°C.

Simultaneous Fixation, Permeabilization and Fluorescent Phallotoxin Staining

The phallotoxin appears to be stable for short periods in 4% formaldehyde fixation buffers. This permits a rapid one-step fixation, permeabilization and labeling procedure as follows.

- 2.1 Prepare a 1 ml solution containing 50 to 100 µg/ml lysopalmitoylphosphatidylcholine and 3.7% formaldehyde and then add 5–10 units of fluorescent phallotoxin (approximately 25 to 50 µl of methanolic stock solution).
- 2.2 Place this staining solution on cells and incubate for 20 minutes at 4°C.
- 2.3 Rapidly wash three times with buffer.
- 2.4 Mount coverslips and view.

Living Cells

Phallotoxins are usually not cell-permeant and have therefore not been used extensively with living cells. However, living cells have been labeled^{11,12}. Pinocytosis appears to be the method of entry for some cells, although hepatocytes “avidly” take up the dye by an unknown mechanism^{5,13}. In general, a larger amount of stain will be needed for staining living cells.

Fluorescence Microscopy

Photostability or resistance to photobleaching is a primary concern when making fluorescence measurements. Alexa Fluor® is significantly more photostable than NBD and fluorescein and will therefore enable more accurate photographic measurements. To further reduce photobleaching, minimize the exposure of fluorescently labeled specimens to light with neutral density filters and expose samples only when observing or recording a signal. Maximize collection of fluorescence by using a minimum of optics, high numerical aperture objectives, relatively low magnification, high-quality optical filters and high-speed film or high-efficiency detectors. Notes [A] Methanol can disrupt actin during the fixation process. Therefore, it is best to avoid any methanol containing fixatives. The preferred fixative is methanol-free formaldehyde.

References

1. Wieland, T. in Phallotoxins, Springer-Verlag, New York (1986);
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