



**Biotin-XX Phalloidin (Cat: 00028)**  
**CF405 Phalloidin (Cat: 00034)**  
**Fluorescein Phalloidin (Cat: 00030)**  
**Rhodamine Phalloidin (Cat: 00027)**  
**Rhodamine 110 Phalloidin (Cat: 00032)**  
**Sulforhodamine 101 (Texas-Red) Phalloidin (Cat: 00033)**

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## Introduction

Phalloidin is a toxin isolated from the deadly *Amanita phalloides* mushroom. It is a bicyclic peptide that binds specifically to F-actin<sup>(1)</sup>. It is a very convenient tool to investigate the distribution of F-actin when labeled with fluorescent dyes. Phalloidin contains an unusual thioether bridge between a cysteine and tryptophan residues that forms an inner ring structure. At elevated pH, this thioether is cleaved and the toxin loses its affinity for actin.

Fluorescent and biotin-conjugated phalloidins are water-soluble and stains F-actin at nanomolar concentrations<sup>(1-3)</sup>. Labeled phalloidins have similar affinity for both large and small filaments, binding in a stoichiometric ratio of about one phalloidin molecule per actin subunit in muscle and nonmuscle cells from various species of plants and animals. Different from antibodies, the binding affinity of phalloidin does not change significantly with actin among different species. Non-specific staining is negligible, and the contrast between stained and unstained areas is extremely large. Phalloidin shifts the monomer/polymer equilibrium toward the polymer, lowering the critical concentration for polymerization up to 30-fold<sup>(3, 4)</sup>. Phallotoxins also stabilize F-actin, inhibiting depolymerization by cytochalasins, potassium iodide and elevated temperatures. Because the phalloidin 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 and troponin can still bind to actin after treatment with phalloidin. Even more significantly, phalloidin-labeled actin filaments remain functional; labeled glycerinated muscle fibers still contract, and labeled actin filaments still move on solid-phase myosin substrates<sup>(5, 6)</sup>. Fluorescent phalloidin can also be used to quantify the amount of F-actin in cells<sup>(7, 8)</sup>.

Fluorescent phalloidins are used to fluorescently label F-actin in cells in a single step. The long flexible spacer group XX of biotin-phalloidin facilitates interaction between biotin and avidin or streptavidin that can be labeled with a fluorophore or an enzyme for visualization of F-actin. Biotin-XX phalloidin can also be used for detecting F-actin by electron microscopy as well.

## Product Components

### ***Biotin-XX Phalloidin (#00028)***

1 vial of 100 units of biotin conjugated Phalloidin

### ***CF405 Phalloidin (#00034)***

1 vial of 300 units of CF405 conjugated Phalloidin

### ***Fluorescein Phalloidin (#00030)***

1 vial of 300 units of fluorescein conjugated Phalloidin

### ***Rhodamine Phalloidin (#00027)***

1 vial of 300 units of rhodamine conjugated Phalloidin

### ***Rhodamine 110 Phalloidin (#00032)***

1 vial of 300 units of rhodamine 110 conjugated Phalloidin

### ***Sulforhodamine 101 (Texas-Red) Phalloidin (#00033)***

1 vial of 300 units of sulforhodamine 101 conjugated Phalloidin

## Spectral Properties of Phallotoxin Probes

Name of the Dye	Ex (nm)	Em (nm)
CF405	405	460
Fluorescein	496	516
Rhodamine	540	565
Rhodamine 110	502	524
Sulforhodamine 101 (Texas-Red)	591	608

## **Storage Conditions**

Upon receipt, these products should be stored in a freezer at  $\leq -20^{\circ}\text{C}$ , desiccated, and protected from light. Once reconstituted in methanol, the stock solutions are stable for at least one year when stored frozen at  $\leq -20^{\circ}\text{C}$ , desiccated, and protected from light.

## **Preparation of Stock Solution**

Each unit of the fluorescent phalloidin is supplied as a lyophilized solid in a vial containing 300 units of the product. The material should be dissolved in 1.5mL methanol to yield a final concentration of 200 units/mL, which is equivalent to approximately 6.6 $\mu\text{M}$ . One unit of fluorescent phalloidin is defined as the amount of material used to stain one microscope slide of fixed cells, according to the following protocol (see below), and is equivalent to 5 $\mu\text{L}$  of methanolic stock solution for each fluorescent phalloidin.

Biotin-XX phalloidin is supplied as a lyophilized solid in a vial containing 100 units of the products. The material should be dissolved in 1 mL methanol to yield a final concentration of 100 units/mL, which is equivalent to approximately 10  $\mu\text{M}$ . Higher concentration of Biotin-XX phalloidin compared to fluorescent phalloidin is needed for staining cells. One unit of fluorescent phalloidin is defined as the amount of material used to stain one microscope slide of fixed cells, according to the following protocol (see below), and is equivalent to 10 $\mu\text{L}$  of methanolic stock solution for biotin-XX phalloidin.

## **Procedures for Staining Slides**

The following protocol describes the staining procedure for adherent cells grown on glass coverslips or 8-well chamber slides.

### **Formaldehyde-Fixed Cells**

1. Wash cells 3 times with chilled PBS.
2. Fix cells on ice with 3.75% formaldehyde solution in PBS (1mL 35% formaldehyde + 9mL chilled PBS) for 15 minutes.
3. Wash 3 times with chilled PBS.
4. Permeabilize cells with 200 $\mu\text{L}$  0.5% Triton X-100 in PBS at room temperature for 10 min.
5. Wash cells 3 times with PBS.
6. Block non-specific binding using 3% non-fat dry milk in PBS at  $4^{\circ}\text{C}$  for 1 hour.
7. Wash cells once with PBS.
8. For fluorescent phalloidin, dilute 5 $\mu\text{L}$  methanolic stock solution of the phalloidin of your choice into 200 $\mu\text{L}$  PBS with 1% BSA for each cover slip or chamber to be stained.

For biotin-XX phalloidin, dilute 10  $\mu\text{L}$  methanolic stock solution of the phalloidin into 200 $\mu\text{L}$  PBS with 1% BSA for each cover slip or chamber to be stained.

9. Place the staining solution on the coverslip for 20 minutes at room temperature (generally, any temperature between  $4^{\circ}\text{C}$  and  $37^{\circ}\text{C}$  is suitable). To avoid evaporation, keep the coverslips inside a covered container and the chamber slides covered during the incubation.
10. Wash 2-3 times with PBS.

11. When using biotin-XX phalloidin, incubate for 30 minutes with 100  $\mu$ L of a 10  $\mu$ g/mL solution of fluorescent or enzyme-conjugated streptavidin dissolved in 100 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.3% Triton X-100 and 1% BSA. Incubate for 15 minutes at room temperature. After incubation, wash with PBS. To develop enzyme activity, follow a procedure recommended for the specific enzyme.
12. For long-term storage, the cells should be air dried and then mounted in a permanent mounting agent 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.

### ***Living Cells***

Phalloidin is usually not cell-permeant and have therefore not been used extensively with living cells. However, living cells have been labeled by pinocytosis or unknown mechanism <sup>(9-12)</sup>. In general, a larger amount of stain will be needed for staining living cells. Alternatively, fluorescent phalloidins have also been injected into cells for monitoring actin distribution and cell motility <sup>(13-16)</sup>.

### ***Notes***

1. The amount of toxin present in a vial could be lethal only to a mosquito (LD<sub>50</sub> of phalloidin = 2 mg/kg), but it should still be handled with caution.
2. 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); **2.** J Muscle Res Cell Motil 9, 370 (1988); **3.** Methods Enzymol 85, 514 (1982); **4.** Eur J Biochem 165, 125 (1987); **5.** Nature 326, 805 (1987); **6.** Proc Natl Acad Sci USA 83, 6272 (1986); **7.** Blood 69, 945 (1987); **8.** Anal Biochem 200, 199 (1992); **9.** J Cell Biol 105, 1473 (1987); **10.** Proc Natl Acad Sci USA 77, 980 (1980); **11.** Nature 284, 405 (1980); **12.** CRC Crit Rev Biochem 5, 185 (1978); **13.** J Cell Biol 106, 1229 (1988); **14.** J Cell Biol 103, 265a (1986); **15.** Eur J Cell Biol 24, 176 (1981); **16.** Proc Natl Acad Sci USA 74, 5613 (1977).