Phalloidins

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Phalloidin is a bicyclic peptide that belongs to a family of toxins isolated from the deadly *Amanita phalloides* mushroom (Wieland, 1986). Fluorescent and biotinylated phalloidins bind F-actin with nanomolar affinity and are water soluble, thus providing convenient probes for labeling, identifying, and quantifying F-actin in cryopreserved tissue sections, cell cultures, or cell-free experiments (Wieland, 1986; Faulstich, 1988; Wang, 1982). Biotin-XX Phalloidin can be used to visualize actin filaments by electron microscopy using standard enzyme-mediated avidin/streptavidin techniques. Unlabeled phalloidins are also available for use as controls in blocking F-actin staining or in promoting actin polymerization.

Due to the small size of phalloidin conjugates (approximate diameter of 12–15 Å and a molecular weight of <2000 daltons), phalloidin-stained actin filaments maintain binding ability to actin-binding proteins, including myosin, tropomyosin, troponin, and DNase I. In addition, phalloidin-stained actin filaments remain functional; labeled glycerinated muscle fibers still contract, and labeled actin filaments still move on solid-phase myosin substrates (Harada, 1987; Kron, 1986).

Alexa Fluor™ Plus phalloidin conjugates are ideal for imaging actin stress fibers, or for use with super-resolution microscopy studies. These phalloidin conjugates retain the same specificity, but demonstrate 3 to 5 times more sensitivity compared to the corresponding Alexa Fluor™ conjugate. A variety of phalloidin conjugates are available, including: Alexa Fluor™ Plus 405 Phalloidin, Alexa Fluor™ Plus 555 Phalloidin, Alexa Fluor™ Plus 647 Phalloidin, and Alexa Fluor™ Plus 750 Phalloidin.

Note: For a complete list of fluorescent or biotinylated phalloidin conjugates, see Table 1 on page 5.

Contents and storage

Contents	Cat. No.	Amount[1]	Storage[2]
Fluorescent phalloidin	See Table 1 on page 5.	1 vial of lyophilized solids (300 units/assay[3])	≤–20°C protected from light
Biotin-XX Phalloidin[4]	B7474	1 vial of lyophilized solids (50 units/assay[3])	
Phalloidin (unlabeled)	P3457	1 vial of lyophilized solids (1 mg)	
For approximate fluorescence excitation and emission (in nm), see Table 1 on page 5.			

[1] The contents of the vial may not be visible.

[2] The product is stable for at least one year when stored as directed.

[3] One unit/assay is defined as the amount of phalloidin that is used to label one microscope slide or coverslip of fixed cells, according to the protocol described in this user guide.

[4] Biotin-XX Phalloidin is conjugated to phalloidin with a carbon linker (XX), which has been shown to enhance the binding ability of conjugated biotin to avidin and streptavidin (Haugland, 1998; Haugland, 1995).

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
DMSO, Anhydrous	D12345
PBS (1X), pH 7.4 (PBS), or equivalent imaging-grade PBS	10010049
Image-iT™ Fixative Solution (4% formaldehyde, methanol-free), or equivalent methanol-free formaldehyde	FB002
Biotin-binding conjugate, such as streptavidin or NeutrAvidin™ fluorescent or enzyme conjugate[1]	MLS
1-Palmitoyl-*sn*-glycero-3-phosphocholine (CAS Number: 17364-16-8)[2]	MLS
(Optional) Triton™ X-100 Surfact-Amps™ Detergent Solution (or equivalent detergent), or imaging-grade acetone	85112
(Optional) Bovine serum albumin (BSA)	MLS
(Optional) Image-iT™ FX Signal Enhancer	I36933
(Optional) BlockAid™ Blocking Solution	B10710
(Optional) DNA counterstain, one of the following, or equivalent: • NucBlue™ Fixed Cell ReadyProbes™ Reagent • SYTOX™ Deep Red Nucleic Acid Stain	• R37606 • S11381
(Optional) ProLong™ Glass Antifade Mountant, or equivalent slide mounting solution	P36980

[1] For use with Biotin-XX Phalloidin only.
[2] Required for the simultaneously fixation, permeabilization, and staining procedure only (see "Simultaneously fix, permeabilize, and stain cells" on page 4).

Procedural guidelines

Handle fluorescent, biotinylated, and unlabeled phalloidins with care although the amount of toxin present in a vial could be lethal only to a mosquito (LD_{50} of phalloidin = 2 mg/kg).

Prepare stock solutions

Prepare fluorescent phalloidins

Prepare a stock solution of fluorescent phalloidins using one of the following methods.

Note: Under standard experimental conditions for cultured cells, preparing a stock solution in anhydrous DMSO yields superior staining intensity and retention of F-actin structural integrity compared to alcohol-based and aqueous solvents.

Method	Procedure
(Recommended) DMSO stock solution	Dissolve the vial contents in 150 µL of anhydrous DMSO to yield a 400X stock solution at a concentration of 2,000 assays/mL, which is equivalent to approximately 66 µM. **Note:** One unit/assay of fluorescent phalloidins is equivalent to 0.5 µL of the DMSO stock solution. The DMSO stock solution is stable for at least one year, when stored at ≤–20°C. This solution has been tested for stability for 5 freeze/thaw cycles. Aliquot the stock solution if additional freeze/thaw cycles are needed.
Methanol stock solution	Dissolve the vial contents in 1.5 mL of methanol to yield a 40X stock solution at a concentration of 2,000 assays/mL, which is equivalent to approximately 66 µM. **Note:** One unit/assay of fluorescent phalloidins is equivalent to 5 µL of the methanolic stock solution.

Prepare Biotin-XX Phalloidin

Note:

- Cells are stained using a higher concentration of Biotin-XX Phalloidin compared to fluorescent phalloidins.
- Cells stained with Biotin-XX Phalloidin require a fluorescent or enzyme-conjugated avidin or streptavidin detection reagent.

Dissolve the vial contents in 0.5 mL of methanol to yield a final concentration of 100 units/assays per mL, which is equivalent to approximately 20 µM.

Note: One unit/assay of Biotin-XX Phalloidin is equivalent to 10 µL of the methanol stock solution.

Prepare unlabeled Phalloidin

Unlabeled Phalloidin is provided as a 1-mg lyophilized solid.

Prepare a stock solution of Phalloidin by dissolving the contents of the vial in the appropriate amount of methanol for your application. It is recommended to prepare the stock solution at a concentration ≤5 mg/mL.

For example, dissolve the vial contents in 200 µL of methanol to yield a 5 mg/mL stock solution, which is equivalent to approximately 6 µM.

Stain formaldehyde-fixed cells

This protocol describes the staining procedure for adherent cells that are grown on glass coverslips.

Note: The staining protocol that is described here is compatible with most signal amplification techniques that are used for ICC, IHC, or FISH (such as Tyramide SuperBoost™ signal amplification).

1. Wash the sample two times with pre-warmed PBS.
2. Fix the sample in 3.7% methanol-free formaldehyde solution in PBS for 15 minutes at room temperature.

   IMPORTANT! Avoid methanol-containing fixatives. Methanol can disrupt actin during the fixation process. We recommend using methanol-free formaldehyde, such as Image-iT™ Fixative Solution (4% formaldehyde, methanol-free) (Cat. No. FB002).
3. Wash the sample two or more times with PBS.
4. Permeabilize the sample in 0.1% Triton™ X-100 in PBS for 15 minutes.

   Note: Certain samples can require permeabilization in an acetone solution at ≤–20°C in a glass petri dish.
5. Wash the sample two or more times with PBS.
6. *(Optional)* When multiplexing with antibodies, incubate the sample in BlockAid™ Blocking Solution (Cat. No. B10710) or a similar blocking solution containing 1% BSA for 30–45 minutes at room temperature .
7. *(Optional)* Incubate the sample in Image-iT™ FX Signal Enhancer (Cat. No. I36933) for 20–30 minutes to enhance the signal.

   Note: If antibody staining is desired, perform the primary and secondary antibody incubation separately, between step 7 and step 8, according to the manufacturer's protocol. Labeling samples with phalloidins can be combined with secondary antibody staining. Ensure that the correct dilutions of each reagent are used in the staining solution.
8. Prepare the staining solution as indicated.

   Note: When staining more than one coverslip, adjust the volumes accordingly. For a stronger signal, use 2–3 times more of the staining solution per coverslip.

For...	Do this...
Fluorescent phalloidin staining solution	1. Dilute the stock solution. • **For a DMSO stock solution—**dilute 0.5 µL of the 400X stock solution in 200 µL of PBS for each coverslip to be stained. • **For a methanol stock solution—**dilute 5 µL of the 40X methanol stock solution in 200 µL of PBS for each coverslip to be stained. 2. Add 1% bovine serum albumin (BSA) to reduce nonspecific background staining.
Biotin-XX Phalloidin staining solution	1. Dilute 10 µL of the methanol stock solution in 200 µL of PBS for each coverslip to be stained. 2. Add 1% bovine serum albumin (BSA) to reduce nonspecific background staining.

9. Incubate the sample in the staining solution as indicated.
   - **For fluorescent phalloidins—**Add the fluorescent phalloidin staining solution to each coverslip, then incubate for 30–60 minutes at room temperature.
     Place the coverslips in a covered container to prevent evaporation during the incubation.
   - **For Biotin-XX Phalloidin—**Add the Biotin-XX Phalloidin staining solution to each coverslip, then incubate for 15 minutes at room temperature.
10. *(Optional)* If needed, add a DNA counterstain, such as NucBlue™ Fixed Cell ReadyProbes™ Reagent (Cat. No. R37606) or SYTOX™ Deep Red Nucleic Acid Stain (Cat. No. S11381) for fixed or dead cells.

11. Wash the sample two or more times with PBS.

    Note: If you are using Biotin-XX Phalloidin, follow the procedure that is recommended for the specific enzyme to develop enzyme activity.

    For example, prepare a 10 µg/mL solution of fluorescent- or enzyme-conjugated streptavidin in 100-mM Tris-HCl (pH 7.5), 150-mM NaCl, 0.3% Triton™ X-100, and 1% BSA. Prepare enough to add 100 µL to each coverslip. Add 100 µL of the fluorescent- or enzyme-conjugated streptavidin solution to each coverslip, then incubate for 30 minutes at room temperature.

12. For long-term storage, mount the sample in a curing/hard-setting aqueous mountant, such as ProLong™ Glass Antifade Mountant (Cat. No. P36980). Specimens that are prepared in this manner retain actin staining for at least six months when stored in the dark at 2–6°C.

    IMPORTANT! Do not use mountants that contain organic solvents.

    Note: If the samples are not mounted, or mounted in non-curing mountants, it is highly recommended to image the cells immediately. Phalloidin conjugates lose their signal intensity with increased storage time. The rate of signal loss during storage can differ depending on the type of conjugate, mountant, or storage temperature used. Our experiments indicate that cells mounted in SlowFade™ Diamond Antifade Mountant or SlowFade™ Glass Antifade Mountant retain actin staining for 2 weeks or more (when stored at −20°C with multiple freeze thaw cycles).

Simultaneously fix, permeabilize, and stain cells

Phalloidins are only stable for a short amount of time in 4% formaldehyde fixation buffers. This protocol describes a rapid one-step fixation, permeabilization, and labeling procedure.

Note: We recommend using the fluorescent phalloidin methanol stock solution for this procedure. For information on preparing the methanol stock solution, see "Prepare fluorescent phalloidins" on page 2.

1. Prepare a 1-mL solution containing 50–100 µg/mL of 1-palmitoyl-*sn*-glycero-3-phosphocholine (CAS No. 17364-16-8) and 3.7% methanol-free formaldehyde, then add 25–50 µL of the fluorescent phalloidin methanol stock solution.
2. Add the solution to the sample, then incubate for 20 minutes at 4°C.
3. Rapidly wash the sample three times with PBS.
4. Mount the coverslip, then image the cells.

Stain live cells

Phalloidins are usually not cell-permeable, however labeling of live cells with phalloidin conjugates has been reported (Barak, 1980; Taylor, 1980). Pinocytosis appears to be the mode of entry for some cells, although hepatocytes efficiently take up phalloidins by an unknown mechanism (Cooper, 1987; Wieland, 1978). In general, a greater amount of phalloidin conjugate is needed for staining live cells. Rhodamine phalloidin has been microinjected into fibroblasts without noticeable changes in shape or ruffling (Cooper, 1988; Snider, 1986). Injections of phalloidin into live cells appear to alter the actin distribution and cell motility (Wehland, 1981; Wehland, 1977).

Consult the literature to find the staining procedure that is appropriate for your experiments.

Supplemental information

Spectral properties of phalloidin conjugates

Labeled phalloidins have similar affinity for large and small filaments, binding in a stoichiometric ratio of one phalloidin molecule per actin subunit in muscle and non-muscle cells from many different species of animals and plants. Unlike antibodies, the binding affinity does not change significantly with actin from different species or sources. Nonspecific staining is negligible, and the contrast between stained and unstained areas is high. It has been reported that phalloidins are unable to bind to monomeric G-actin (Wieland, 1986). Phalloidins shift the monomer/polymer equilibrium toward the polymer and lower the critical concentration for polymerization up to 30-fold (Wang, 1982; Miki, 1987). Phalloidins also stabilize F-actin, inhibiting depolymerization by cytochalasins, potassium iodide, and elevated temperatures.

The approximate molecular weight (MW) of unlabeled phalloidin is 790 daltons. See Table 1 for the approximate MWs, and the peak excitation and emission wavelengths for each phalloidin conjugate.

Table 1 Spectral characteristics and dissociation constants of phalloidin probes

Cat. No.	Amount	Conjugate	Excitation[1]	Emission[1]	Approximate MW
A22281	300 U	Alexa Fluor™ 350 Phalloidin	346	442	1100 Da
A30104	300 U	Alexa Fluor™ Plus 405 Phalloidin	405	450	1010 Da
A12379	300 U	Alexa Fluor™ 488 Phalloidin	495	518	1320 Da
F432	300 U	Fluorescein Phalloidin	496	516	1175 Da
O7466	300 U	Oregon Green™ 488 Phalloidin	496	520	1180 Da
O7465	300 U	Oregon Green™ 514 Phalloidin	511	528	1281 Da
A22282	300 U	Alexa Fluor™ 532 Phalloidin	531	554	1350 Da
R415	300 U	Rhodamine Phalloidin	540	565	1250 Da
A22283	300 U	Alexa Fluor™ 546 Phalloidin	556	570	1800 Da
A34055	300 U	Alexa Fluor™ 555 Phalloidin	555	565	1910 Da
A30106	300 U	Alexa Fluor™ Plus 555 Phalloidin	555	565	1488 Da
B3475	300 U	BODIPY™ 558/568 Phalloidin	558	569	1115 Da
A12380	300 U	Alexa Fluor™ 568 Phalloidin	578	600	1590 Da
A12381	300 U	Alexa Fluor™ 594 Phalloidin	581	609	1620 Da
T7471	300 U	Texas Red™-X Phalloidin	591	608	1490 Da
A22284	300 U	Alexa Fluor™ 633 Phalloidin	632	647	1900 Da
A34054	300 U	Alexa Fluor™ 635 Phalloidin	633	647	1850 Da
A22287	300 U	Alexa Fluor™ 647 Phalloidin	650	668	1950 Da
A30107	300 U	Alexa Fluor™ Plus 647 Phalloidin	650	668	1514 Da
A22285	300 U	Alexa Fluor™ 660 Phalloidin	663	690	1750 Da
A22286	300 U	Alexa Fluor™ 680 Phalloidin	679	702	1850 Da
A30105	300 U	Alexa Fluor™ Plus 750 Phalloidin	758	784	2122 Da
B7474	50 U	Biotin-XX Phalloidin	NA	NA	1300 Da
P3457	1 mg	Phalloidin (unlabeled)	NA	NA	790 Da

[1] Approximate fluorescence excitation and emission maxima, in nm. Go to **www.thermofisher.com** for complete spectra information.

Related products

Cat. No.	Product name	Amount
P36980	ProLong™ Glass Antifade Mountant	5 × 2 mL
P36981	ProLong™ Glass Antifade Mountant with NucBlue™ (Hoechst 33342)	5 × 2 mL
P36992	ProLong™ Glass Antifade Mountant with SYTOX™ Deep Red Nucleic Acid Stain	5 × 2 mL

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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Revision	Date	Description
B.0	10 October 2019	Added new fluorescent phalloidins (Cat. No. A30106 and A30107), updated required materials not supplied, updated the instructions for preparation of stock solutions, added instructions for samples that are not mounted, and updated related product information.
A.0	13 June 2018	Changed title from Phallotoxins to Phalloidins, added Cat. Nos. A30104 and A3015, removed discontinued products, updated protocol for staining formadehyde-fixed cells, rebranded, and updated legal and regulatory language.
1.0	4 January 2006	Baseline for this revision.