# Phalloidin and Phalloidin Conjugates for Staining Actin Filaments

## Introduction

Actin is a globular, roughly 42-kDa protein found in almost all eukaryotic cells. It is also one of the most highly conserved proteins, differing by no more than 20% in species as diverse as algae and humans. Actin is the monomeric subunit of two types of filaments in cells: microfilaments, one of the three major components of the cytoskeleton, and thin filaments, part of the contractile apparatus in muscle cells. Thus, actin participates in many important cellular processes including muscle contraction, cell motility, cell division and cytokinesis, vesicle and organelle movement, cell signaling, as well as the establishment and maintenance of cell junctions and cell shape.

Our phalloidin conjugates selectively binds to F-actins. Used at nanomolar concentrations, phalloidin derivatives are convenient probes for labeling, identifying and quantitating F-actins in formaldehyde-fixed and permeabilized tissue sections, cell cultures or cell-free experiments. Phalloidin binds to actin filaments much more tightly than to actin monomers, leading to a decrease in the rate constant for the dissociation of actin subunits from filament ends, essentially stabilizing actin filaments through the prevention of filament depolymerization. Moreover, phalloidin is found to inhibit the ATP hydrolysis activity of F-actin. Phalloidin functions differently at various concentrations in cells. When introduced into the cytoplasm at low concentrations, phalloidin recruits the less polymerized forms of cytoplasmic actin as well as filamin into stable "islands" of aggregated actin polymers, yet it does not interfere with stress fibers, i.e. thick bundles of microfilaments. The property of phalloidin is a useful tool for investigating the distribution of F-actin in cells by labeling phalloidin with fluorescent analogs and using them to stain actin filaments for light microscopy. Fluorescent derivatives of phalloidin have turned out to be enormously useful in localizing actin filaments in living or fixed cells as well as for visualizing individual actin filaments in vitro. Fluorescent phalloidin derivatives have been used as an important tool in the study of actin networks at high resolution. AAT Bioquest offers a variety of fluorescent phalloidin derivatives with different colors for multicolor imaging applications as summarized in the following table.

## Spectral Properties

The excitation and emission maxima of the phalloidin conjugates are summarized in the following table.

| Cat. # | Product Name | Unit | MW | Ex (nm) | Em (nm) |
|---|---|---|---|---|---|
| 5301 | Phalloidin | 1 mg | 788.88 | N/A | N/A |
| 5302 | Phalloidin Amine | 100 µg | 901.91 | N/A | N/A |
| 23100 | Phalloidin-AMCA Conjugate | 300 tests | ~1000 | 353 | 442 |
| 23101 | Phalloidin-Fluorescein Conjugate | 300 tests | ~1100 | 492 | 518 |
| 23102 | Phalloidin-Tetramethylrhodamine Conjugate | 300 tests | ~1200 | 546 | 575 |
| 23103 | Phalloidin-California Red Conjugate* | 300 tests | ~1000 | 583 | 605 |
| 23110 | Phalloidin-iFluor™ 350 Conjugate | 300 tests | ~1000 | 353 | 442 |
| 23111 | Phalloidin-iFluor™ 405 Conjugate | 300 tests | ~1400 | 400 | 421 |
| 23115 | Phalloidin-iFluor™ 488 Conjugate | 300 tests | ~1900 | 493 | 517 |
| 23116 | Phalloidin-iFluor™ 514 Conjugate | 300 tests | ~1800 | 520 | 547 |
| 23117 | Phalloidin-iFluor™ 532 Conjugate | 300 tests | ~1800 | 542 | 558 |
| 23119 | Phalloidin-iFluor™ 555 Conjugate | 300 tests | ~1300 | 556 | 574 |
| 23122 | Phalloidin-iFluor™ 594 Conjugate | 300 tests | ~1600 | 590 | 618 |
| 23125 | Phalloidin-iFluor™ 633 Conjugate | 300 tests | ~2100 | 634 | 649 |
| 23127 | Phalloidin-iFluor™ 647 Conjugate | 300 tests | ~2200 | 650 | 665 |
| 23128 | Phalloidin-iFluor™ 680 Conjugate | 300 tests | ~2700 | 681 | 698 |
| 23129 | Phalloidin-iFluor™ 700 Conjugate | 300 tests | ~3000 | 692 | 708 |
| 23130 | Phalloidin-iFluor™ 750 Conjugate | 300 tests | ~3300 | 752 | 778 |
| 23131 | Phalloidin-iFluor™ 790 Conjugate | 300 tests | ~2800 | 787 | 808 |

*Excellent replacement to Texas Red-phalloidin conjugate due to their essentially identical spectral properties.


**Ordering:** sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
**Technical Support:** support@aatbio.com; Tel: 408-733-1055

## Storage and Handling Conditions

The fluorescent phalloidin conjugates are either 1000X stock solution in DMSO or lyophilized powder. Both the solutions and powders should be stable for at least 6 months if store at -20 °C. Protect the fluorescent conjugates from light, and avoid freeze/thaw cycles.

*Note: Phalloidin is toxic, although the amount of toxin present in a vial could be lethal only to a mosquito (LD50 of phalloidin = 2 mg/kg), it should be handled with care.*

## Assay Protocol

**Brief Summary**

**Prepare samples in microplate wells → Remove liquid from samples in the plate → Add phalloidin-iFluor™ solution (100 µL/well) → Stain the cells at RT for 20 to 90 minutes → Wash the cells → Examine the specimen under microscope**

*Note: Warm the vial to room temperature and centrifuge briefly before opening.*

**1. Prepare 1000 X Phalloidin DMSO stock solution:** by adding 30 µL of DMSO into the powder form vials.

**2. Prepare 1X Phalloidin conjugate working solution:** by adding 1 µL of 1000X Phalloidin conjugate DMSO solution to 1 mL of PBS with 1% BSA.
*Note 1: The unused 1000X DMSO stock solution of phalloidin conjugate should be aliquoted and stored at -20°C. protected from light.*
*Note 2: Different cell types might be stained differently. The concentration of phalloidin conjugate working solution should be prepared accordingly.*

**3. Stain the cells:**

2.1 Perform formaldehyde fixation. Incubate cells with 3.0–4.0 % formaldehyde in PBS at room temperature for 10–30 minutes.
*Note: Avoid any methanol containing fixatives since methanol can disrupt actin during the fixation process. The preferred fixative is methanol-free formaldehyde.*

2.2 Rinse the fixed cells 2–3 times in PBS.

2.3 Optional: Add 0.1% Triton X-100 in PBS into fixed cells (from Step 2.2) for 3 to 5 minutes to increase permeability. Rinse the cells 2–3 times in PBS.

2.4 Add 100 µL/well (96-well plate) of phalloidin conjugate working solution (from Step 1) into the fixed cells (from Step 2.2 or 2.3), and stain the cells at room temperature for 20 to 90 minutes.

2.5 Rinse cells gently with PBS 2 to 3 times to remove excess phalloidin conjugate before plating, sealing and imaging under microscope.

## References

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2. Johnson S C, Nancy M. McKenna M N, and Wang Y (1988). Association of microinjected myosin and its subfragments with myofibrils in living muscle cells. J Cell Biol, 107(6), 2213.
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4. Miki M, Barden JA, dos Remedios CG, Phillips L, Hambly BD (1987). Interaction of phalloidin with chemically modified actin. Eur J Biochem 165, 125.
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