

## 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

Table 1: The excitation and emission maxima of the phalloidin conjugates.

Cat. #	Product Name	Unit	MW	Forms	Ex (nm)	Em (nm)
5301	Phalloidin	1 mg	788.88	Solid	N/A	N/A
5302	Phalloidin Amine	100 µg	901.91	Solid	N/A	N/A
23100	Phalloidin-AMCA Conjugate	300 tests	~1000	Liquid	353	442
23101	Phalloidin-Fluorescein Conjugate	300 tests	~1100	Liquid	492	518
23102	Phalloidin-Tetramethylrhodamine Conjugate	300 tests	~1300	Liquid	546	575
23103	Phalloidin-California Red Conjugate*	300 tests	~1000	Liquid	583	605
23110	Phalloidin-iFluor™ 350 Conjugate	300 tests	~1300	Liquid	353	442
23111	Phalloidin-iFluor™ 405 Conjugate	300 tests	~1400	Liquid	400	421
23115	Phalloidin-iFluor™ 488 Conjugate	300 tests	~1900	Liquid	493	517
23116	Phalloidin-iFluor™ 514 Conjugate	300 tests	~1800	Liquid	520	547
23117	Phalloidin-iFluor™ 532 Conjugate	300 tests	~1800	Liquid	531	556
23119	Phalloidin-iFluor™ 555 Conjugate	300 tests	~1300	Liquid	556	574
23122	Phalloidin-iFluor™ 594 Conjugate	300 tests	~1600	Liquid	590	618
23125	Phalloidin-iFluor™ 633 Conjugate	300 tests	~1700	Solid	634	649
23127	Phalloidin-iFluor™ 647 Conjugate	300 tests	~1600	Solid	650	665
23128	Phalloidin-iFluor™ 680 Conjugate	300 tests	~2000	Solid	681	698
23129	Phalloidin-iFluor™ 700 Conjugate	300 tests	~3000	Solid	692	708
23130	Phalloidin-iFluor™ 750 Conjugate	300 tests	~3300	Solid	752	778
23131	Phalloidin-iFluor™ 790 Conjugate	300 tests	~2800	Solid	787	808
23150	AF350 Phalloidin [equivalent to Alexa Fluor® 350 phalloidin]	300 tests	~1100	Liquid	346	445
23153	AF488 Phalloidin [equivalent to Alexa Fluor® 488 phalloidin]	300 tests	~1300	Liquid	494	517
23158	AF594 Phalloidin [equivalent to Alexa Fluor® 594 phalloidin]	300 tests	~1600	Liquid	590	617

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

### Storage and Handling Conditions

The fluorescent phalloidin conjugates are either 1000X stock solution in DMSO (liquid form) or lyophilized powder (see Table 1). 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:**

- 3.1 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.*
- 3.2 Rinse the fixed cells 2–3 times in PBS.
- 3.3 **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.
- 3.4 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.
- 3.5 Rinse cells gently with PBS 2 to 3 times to remove excess phalloidin conjugate before plating, sealing and imaging under microscope.

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