Phalloidins Alexa Fluor conjugated

Cat. N°	Description	Ex/Em(nm)	Size
PP-10052	Phalloidin Alexa Fluor 488 conj.	490nm/515nm	300 U
PP-10053	Phalloidin Alexa Fluor 555 conj.	555nm/565nm	300 U
PP-10055	Phalloidin Alexa Fluor 594 conj.	593nm/614 nm	300 U
PP-10057	Phalloidin Alexa Fluor 647 conj.	650nm/665nm	300 U
PP-10059	Phalloidin TRITC conj.	540～546nm/565～575nm	300 U

Applications

- Preparation of fluorescent actin filaments in vitro
- Fluorescent staining of fixed cells and frozen tissues
- Compatible with super-resolution microscopy, confocal microscopy, STORM, SMLM, SIM

Background:

Phalloidin is a seven amino acid peptide toxin from the mushroom Amanita phalloides, which binds specifically and with high affinity (Kd 20 nM) to the polymerized form of actin (F-actin). Phalloidin lowers the critical concentration of actin polymerization to less than 1 μg/ml, thereby acting as a polymerization enhancer

Phalloidin conjugates are commonly used in fluorescence imaging to selectively stain F-actin across a range of sample types, including fixed cells, tissue sections, and cell-free systems

PROTOCOL

1. PREPARATION OF WORKING SOLUTION IN DMSO

1) Stock preparation: Prepare 1000x stock solution by dissolving lyophilized phalloidin (300T) in 30uL DMSO.
This stock solution can be sub-packaged then stored at -20°C.
Solutions should be prepared **fresh** and **protected from light** whenever possible.
2）Working solution：Prepare 1x working solution by adding 1 μL of AlexaFluor -Phalloidin stock solution to 1 mL of PBS with 1% BSA.
Note: The stock solution of phalloidin conjugate should be aliquoted and stored at -20 °C. protected from light.
Note: Different cell types might be stained differently. The concentration of phalloidin conjugate working solution should be prepared accordingly.

Preparing culture of fixed adherent cells

2.1 Grow cells in a 96 well black wall/clear bottom plate until they reach confluence (70–80%).

2.2 Cells can also be grown directly on coverslips inside a petri dish.

2.3 Aspirate cell culture medium (with care to avoid dislodging cells).

2.4 Wash once in PBS.

- ✓ **Tip:** Avoid fixatives containing methanol or acetone: these disrupt the actin structure and prevent phalloidin staining.
- ✓ **Tip:** Suspension cells can be attached to poly-D-lysine microplates or coverslips and then stained using the protocol for adherent cells.

Tip: Avoid fixatives containing methanol or acetone: these disrupt the actin structure and prevent phalloidin staining.

Alternative step: Preparing culture of cells in suspension

2.1 Grow cells until they reach desired confluence (70–80%).

2.2 Centrifuge cells at 1,000 rpm for 5 minutes and aspirate the supernatant, preserving the cell pellet.

2.3 Resuspend the cell pellets gently in pre-warmed (37°C) growth medium and transfer to microplate or coverslips.

2.4 Aspirate cell culture medium carefully to avoid dislodging cells. Wash once in PBS.

- ✓ **Tip:** If you need to save time, suspension cells can be attached to poly-D-lysine microplates or coverslips and then stained using the protocol for adherent cells.

Staining cultured / fixed cells and frozen tissues

Tip: Pre-incubating fixed cells with 1% BSA in PBS for 20–30 minutes may improve staining.
Tip: When staining coverslips, keep them in a covered container to minimize evaporation.
3.1 Fix cells in 3–4% formaldehyde in PBS at room temperature for 10–30 minutes.
3.2 Aspirate fixation solution and wash cells 2–3 times in PBS.

- ✓ **Tip:** Quench excess formaldehyde with 10 mM ethanolamine in PBS (or 0.1 M glycine in PBS) for 5 min.
- ✓ **Tip:** Add 0.1% Triton X-100 in PBS into the fixed cells for 3–5 minutes to increase permeability. Then wash cells 2–3 times in PBS.
- ✓ **Tip:** If cells do not appear healthy, add serum (2–10% range) to stain and wash solutions.

3.3 Add 100 µL/well (96-well plate) phalloidin-conjugate working solution. Incubate at room temperature for 20–90 minutes.

- ✓ **Tip:** add DNA staining dye at this point.
- ✓ **Tip:** for vertebrate cells, it may be possible to add phalloidin-conjugate to the final PBS wash and mount it in that medium.

3.4 To remove excess phalloidin conjugate， Rinse cells 2–3 times with PBS, 5 min per wash .

3.5 Add mounting media to preserve fluorescence (and seal to the slide if using coverslips).
3.6 Imaging under microscope with the proper filter :

Tip: A fast one-step approach to phalloidin staining is effective in some circumstances: a 20-minute incubation at 4ºC in 3.7% formaldehyde and 50–100 µg/mL lysopalmitoyl phosphatidylcholine with phalloidin conjugate, followed by three washes and mounting.

NOTE: Always wear lab coats, gloves and goggles when working with our products although they are low-risk chemicals for R&D only.

Note: Phalloidins Alexa Fluor conj. are not suitable for paraffin embedded tissues and in vivo cells.