# Phalloidin - TRITC

# User Manual

**Catalog # CRG1039**

Phalloidin conjugates are convenient probes for labeling, identifying and quantifying animal or plant actin filaments in formaldehyde-fixed, permeabilized tissue sections, cell cultures or cellfree experiments.

**For research use only. Not for diagnostic or therapeutic procedures.**

## I. INTRODUCTION

Phalloidin is a bicyclic peptide that belongs to a family of toxins isolated from the deadly Amanita phalloides mushroom. Fluorescent 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. Phalloidin contains an unusual thioether bridge between 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. Fluorescently labeled phalloidins stain F-actin at nanomolar concentrations. 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 non-muscle 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. Phallotoxins also stabilize F-actin, inhibiting depolymerization by cytochalasin, potassium iodide and elevated temperatures. Because the phalloidin conjugates are small, with an approximate diameter of 12-15Å 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. Fluorescent phalloidin can also be used to quantify the amount of F-actin in cells.

## II. MATERIALS REQUIRED BUT NOT PROVIDED

1. PBS (1X)
2. 4% Formaldehyde
3. 0.5% Triton X-100

## III. 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 (LD50 of phalloidin = 2 mg/kg).

## IV. WORKING SOLUTION PREPARATION

**Stock Solution**: Dissolve the lyophilized powder in 300 µl PBS for the 300 Assays size or 50 µl PBS for the 50 Assays size.

Dilute 1 µl fluorescent phalloidin stock solution in 200 µl PBS before use.
(For fluorescent phalloidins, the recommended dilution ratio is 1:40 - 1:200, one time experiment is equivalent to 1-5 µl stock solution in a total staining volume of 200 µl.)
*Note*: The dilution ratio can be adjusted appropriately according to the experimental effect.

## V. ASSAY PROCEDURE

**Staining fixed cells**

The following protocol describes the staining procedure for adherent cells grown on glass coverslips or 8-well chamber slides. Phalloidins also can be used to stain fixed frozen or paraffin tissue sections, as well as yeast and fungi.

1. Wash cells 3 times with PBS.
2. Fix cells on ice with 4% formaldehyde solution in PBS for 15 minutes.

Note: Methanol can disrupt actin during the fixation process. Therefore, it is best to avoid any methanol containing fixatives or other solvent-based fixatives. The preferred fixative is methanol-free formaldehyde.

3. Wash cells 3 times with PBS.
4. Permeabilize cells with 0.5% Triton X-100 in PBS at room temperature for 10 minutes.
5. Wash cells 3 times with PBS.
6. Dilute 1-5 µl fluorescent phalloidin stock solution in 200 µl PBS for each cover slip or chamber to be stained. Place the staining solution on the coverslip for 20 minutes at room temperature.

*Note*：Staining volume can be adjusted according to the sample. To avoid evaporation, keep the coverslips inside a covered container and the chamber slides covered during the incubation.

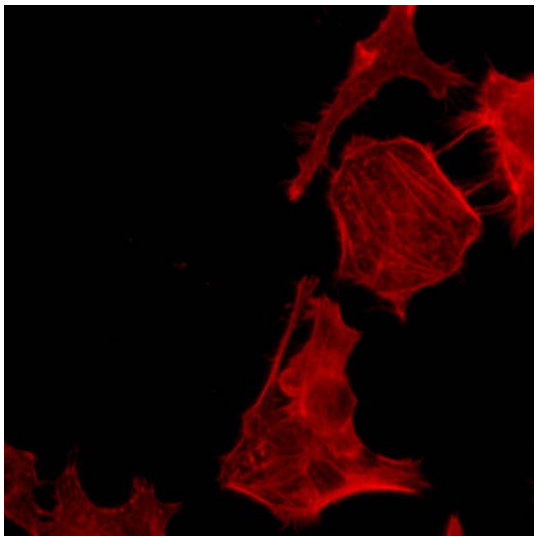
7. Wash 2-3 times with PBS.
8. Image using fluorescence microscopy. Fluorescent phalloidins are photostable enough to image in PBS, but for best results we recommend mounting with antifade mounting medium.

**Staining living cells**

Fluorescently-labeled phalloidin is not cell-permeant and has therefore has not been used extensively with living cells. However, living cells have been labeled by pinocytosis or unknown mechanism. 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.

| Conjugate | Excitation | Emission |
|---|---|---|
| Phalloidin - AF350 | 347 nm | 448 nm |
| Phalloidin - AF405 | 404 nm | 431 nm |
| Phalloidin - AF488 | 491 nm | 512 nm |
| Phalloidin - AF532 | 525 nm | 554 nm |
| Phalloidin - AF555 | 555 nm | 565 nm |
| Phalloidin - AF568 | 575 nm | 598 nm |
| Phalloidin - AF594 | 593 nm | 614 nm |
| Phalloidin - AF633 | 630 nm | 650 nm |
| Phalloidin - AF647 | 648 nm | 664 nm |
| Phalloidin - AF660 | 663 nm | 682 nm |
| Phalloidin - AF680 | 681 nm | 698 nm |
| Phalloidin - AF750 | 750 nm | 777 nm |
| Phalloidin - FITC | 496 nm | 516 nm |
| Phalloidin - TRITC | 545 nm | 570 nm |

**Table 1** Spectral characteristics and dissociation constants of phalloidin probes



Actin filaments (red) were stained with Phalloidin - TRITC

## VI. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

## VII. NOTES