



Phalloidin - AcalephFluor488

User Manual

Catalog # CRG1033

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.....2

II. MATERIALS REQUIRED BUT NOT PROVIDED.....3

III. PROCEDURAL GUIDELINES.....3

IV. WORKING SOLUTION PREPARATION.....4

V. ASSAY PROCEDURE.....5

VI. TECHNICAL SUPPORT.....7

VII. NOTES.....7

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. Phalloidins 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 μ l PBS for the 300 Assays size or 50 μ l PBS for the 50 Assays size.

Dilute 1 μ l fluorescent phalloidin stock solution in 200 μ l PBS before use.

(For fluorescent phalloidins, the recommended dilution ratio is 1:40 - 1:200, one time experiment is equivalent to 1-5 μ l stock solution in a total staining volume of 200 μ l.)

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 μ l fluorescent phalloidin stock solution in 200 μ l 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.

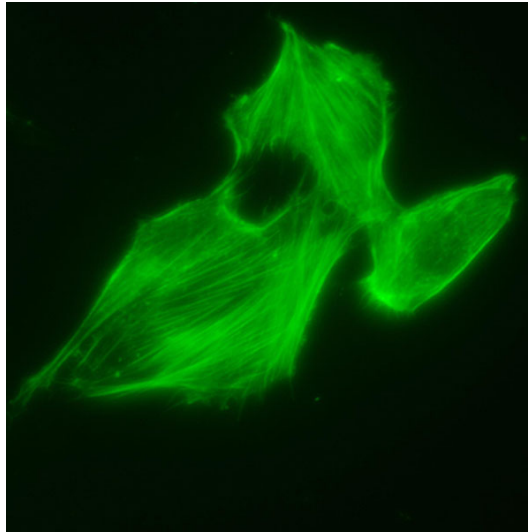
7. Wash 2-3 times with PBS.
8. Image using fluorescence microscopy. Fluorescent phalloidins are photostable enough to image in PBS, but for best results we recommend mounting with antifade mounting medium.

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 - AcalephFluor350	347 nm	448 nm
Phalloidin - AcalephFluor405	404 nm	431 nm
Phalloidin - AcalephFluor488	491 nm	512 nm
Phalloidin - AcalephFluor532	525 nm	554 nm
Phalloidin - AcalephFluor555	555 nm	565 nm
Phalloidin - AcalephFluor568	575 nm	598 nm
Phalloidin - AcalephFluor594	593 nm	614 nm
Phalloidin - AcalephFluor633	630 nm	650 nm
Phalloidin - AcalephFluor647	648 nm	664 nm
Phalloidin - AcalephFluor660	663 nm	682 nm
Phalloidin - AcalephFluor680	681 nm	698 nm
Phalloidin - AcalephFluor750	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 (green) were stained with Phalloidin - AcalephFluor488

VI. TECHNICAL SUPPORT

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

VII. NOTES