Phalloidin - FITC

Cat #: orb1289368 (manual)

For Research Use Only. Not For Use in Diagnostic Procedures!

Product Features

CAS Number: 915026-99-2

MW: 1177.27

Formula: $C_{56}H_{60}N_{10}O_{15}S_2$

Sample Types: Formaldehyde-fixed, permeabilized tissue sections, cell cultures or cell free experiments

Form: Lyophilized powder. Please centrifuge before use, then add an appropriate solvent to dissolve. The solution after reconstitution is nearly transparent.

Introduction

Phalloidin is a bicyclic peptide belonging to a family of toxins isolated from the deadly mushroom Amanita phalloides. Fluorescent phalloidins bind F-actin with nanomolar affinity and are water-soluble, making them convenient probes for labelling, identifying, and quantifying F-actin in cryopreserved tissue sections, cell cultures, and cell-free systems. Phalloidin contains an unusual thioether bridge between cysteine and tryptophan residues, forming an inner ring structure. At elevated pH, this thioether bond is cleaved, resulting in loss of affinity for actin. Fluorescently labelled phalloidins stain F-actin at nanomolar concentrations and exhibit similar affinity for both large and small filaments, binding in a stoichiometric ratio of approximately one phalloidin molecule per actin subunit in both muscle and non-muscle cells across various plant and animal species. Unlike antibodies, phalloidin binding affinity is not significantly affected by species differences in actin. Nonspecific staining is negligible, resulting in high contrast between stained and unstained areas. Phalloidin shifts the monomer–polymer equilibrium toward the polymer, reducing the critical concentration for polymerization by up to 30-fold. Phallotoxins also stabilize F-actin, inhibiting depolymerization induced by cytochalasin, potassium iodide, and elevated temperatures.

Due to their small size (approximately 12–15 Å in diameter and <2000 Da in molecular weight), phalloidin conjugates do not prevent binding of actin-associated proteins such as myosin, tropomyosin, and troponin. Phalloidin-labelled actin filaments remain functional: labelled glycerinated muscle fibres retain contractility, and labelled actin filaments can still move on solid-phase myosin substrates. Fluorescent phalloidin can also be used to quantify F-actin levels in cells.

Materials Required but Not Provided

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

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Procedural Guidelines

Handle fluorescent, biotinylated, and unlabelled phalloidins with care although the amount of toxin present in a vial. (LD50 of phalloidin = 2 mg/kg).

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 of fluorescent phalloidin stock solution in 200 µL PBS before use.

(For fluorescent phalloidins, the recommended dilution range is 1:40–1:200. A typical experiment uses 1–5 µL of stock solution in a total staining volume of 200 µL.)

Note: The dilution ratio can be adjusted as needed based on experimental result.

Assay Procedure

Staining Instructions

Staining Fixed Cells

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

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

Note: Methanol can disrupt actin during fixation. Therefore, methanol-containing fixatives or other solvent-based fixatives should be avoided. Methanol-free formaldehyde is recommended.

3. Wash cells three times with PBS.
4. Permeabilize cells with 0.5% Triton X-100 in PBS at room temperature for 10 minutes.
5. Wash cells three times with PBS.
6. Dilute 1–5 µL of fluorescent phalloidin stock solution in 200 µL PBS for each coverslip or chamber to be stained. Apply the staining solution to the coverslip and incubate for 20 minutes at room temperature.

Note: The staining volume can be adjusted according to the sample. To prevent evaporation, keep coverslips in a covered container and chamber slides covered during incubation.

7. Wash 2–3 times with PBS.
8. Image using fluorescence microscopy. Fluorescent phalloidins are sufficiently photostable for imaging in PBS; however, for optimal results, mounting with an antifade mounting medium is recommended.

Staining Living Cells

Fluorescently labeled phalloidin is not cell-permeant and therefore has not been widely used for staining living cells. However, living cells have been labeled through pinocytosis or other unknown mechanisms. In general, higher amounts of stain are required for staining living cells. Alternatively, fluorescent phalloidins can be injected into cells to monitor actin distribution and cell motility.

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Table 1 Spectral characteristics and dissociation constants of phalloidin probes

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

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