Product Information

Phalloidin Conjugates

See product page for a full list of product names, unit sizes, and catalog numbers.

Storage and Handling

Store at -20°C, desiccated and protected from light. Lyophilized product is stable for at least one year from date of receipt when stored as recommended. After reconstitution, stock solutions are stable for at least one year when stored at -20°C. See Experimental Protocols for detailed directions on reconstitution.

Important: See notes about the compatibility of specific CF® Dyes with fluorescence mounting media, and about the stability of phalloidin staining, after step 10 in the staining fixed cells protocol.

Product Technical Information

See product page for spectral properties and other dye-specific technical information. See our Spectra Viewer to view and download the dye excitation and emission spectra.

Product Description

Phalloidin is a toxin isolated from the deadly *Amanita phalloides* mushroom. It is a bicyclic peptide that binds specifically to F-actin (1). It is a very convenient tool to investigate the distribution of F-actin when labeled with fluorescent dyes. 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 (1-3). 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. Unlike 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 of actin toward the polymer, lowering the critical concentration for polymerization up to 30-fold (3,4). Phallotoxins also stabilize F-actin by inhibiting depolymerization caused by cytochalasins, potassium iodide, and elevated temperatures. Because the phalloidin conjugates are small, with an appropriate diameter of 12-15 Å and molecular weight of <2,000 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 (5,6). Fluorescent phalloidin can also be used to quantify the amount of F-actin in cells (7,8).

Biotium offers phalloidin conjugated to a wide selection of CF® Dyes and other labels. CF® Dyes are a series of next-generation fluorescent dyes developed at Biotium to have combined advantages in brightness, photostability, and water solubility compared to other fluorescent dyes.

Experimental Protocols

Reconstitution

CF® Dye Phalloidin Conjugates: Dissolve the lyophilized solid in methanol or water (1.5 mL for the 300 U size or 0.25 mL for the 50 U size) to yield a stock solution of 200 U/mL.

Other fluorescent Phalloidin Conjugates: Dissolve 300 U lyophilized solid in 1.5 mL methanol to yield a stock solution of 200 U/mL (approximately 6.6 uM).

Biotin-XX-Phalloidin: Dissolve 100 U lyophilized solid in 1 mL methanol to yield a stock solution of 100 U/mL (approximately 10 uM).

One unit (U) of fluorescent phalloidin is defined as the amount of material used to stain one microscope slide of fixed cells.

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. Phalloidin staining is compatible with upstream or downstream immunofluorescence staining. Phalloidin staining is generally performed after immunolabeling.

Note: When staining yeast in liquid culture, cells in log phase stain much better than cells in stationary phase.

Materials required but not provided

- PBS
- Paraformaldehyde, 4% in PBS (Cat. No. 22023)
- Triton® X-100

Staining procedure for fixed cells

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

   Note: Methanol or any other solvent will disrupt actin during the fixation process. The preferred fixative is methanol-free formaldehyde.
3. Wash cells 3 times with PBS.
4. Permeabilize cells with 0.1-0.5% Triton® X-100 in PBS at room temperature for 10 minutes.
5. Wash cells 3 times with PBS.
6. Dilute 5 uL fluorescent phalloidin stock solution in 200 uL PBS for each cover slip or chamber to be stained. For Biotin-XX-Phalloidin, dilute 10 uL stock solution in 200 uL PBS. Volumes can be scaled as necessary depending on the size of the specimen or culture vessel.

   Note: For staining yeast or fungi, increasing the phalloidin concentration from 5 U/mL to 50 U/mL may improve penetration into the cells.
7. Place the staining solution on the cells for 20 minutes at room temperature. To avoid evaporation, keep the coverslips inside a covered container and the chamber slides covered during the incubation.
8. Wash cells 2-3 times with PBS.
9. For Biotin-XX-Phalloidin, continue with biotin detection using labeled streptavidin or anti-biotin antibody (see Related Products). For fluorescent phalloidins, proceed to imaging.
10. CF® Dye Phalloidins can be imaged in PBS, but for best results, especially for preserving staining long-term, we recommend mounting with EverBrite™ antifade mounting media (see Related Products).

    Notes:

    a. CF®647, CF®660C, and CF®680 are cyanine-based dyes and are not compatible with VECTASHIELD® mounting media (Vector Labs). Biotium's EverBrite™ antifade mounting media (see Related Products) are compatible with a wide-range of fluorescent dyes, including cyanine dyes and CF® Dyes.

    b. Fluorescent dyes can affect the stability of phalloidin staining which can cause loss of signal intensity over time. If samples are not mounted, it is highly recommended to image the cells immediately. For best results, store phalloidin-stained samples in a suitable mounting medium at 4°C, protected from light.

    c. For certain phalloidin conjugates, especially CF®543, CF®647, and CF®680, we recommend imaging immediately or shortly after staining. CF®647 and CF®680 phalloidins are recommended for STORM applications, but due to the instability of staining with these conjugates, we do not recommend using them for other microscopy applications. Staining with other CF® Dye Phalloidin Conjugates is more stable and signal could last for several days when specimens are stored at 4°C, protected from light.

Staining living cells

Fluorescently-labeled phalloidin is not cell-permeant and has therefore not been used extensively with living cells. However, living cells have been labeled by pinocytosis or unknown mechanisms (9-12). 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 (13-16).

References

1. Springer-Verlag New York 47 (1986); 2. J Muscle Res Cell Motil 9, 370 (1988); 3. Methods Enzymol 85, 514 (1982); 4. Eur J Biochem 165, 125 (1987); 5. Nature 326, 805 (1987); 6. Proc Natl Acad Sci USA 83, 6272 (1986); 7. Blood 69, 945 (1987); 8. Anal Biochem 200, 199 (1992); 9. J Cell Biol 105, 1473 (1987); 10. Proc Natl Acad Sci USA 77, 980 (1980); 11. Nature 284, 405 (1980); 12. CRC Crit Rev Biochem 5, 185 (1978); 13. J Cell Biol 106, 1229 (1988); 14. J Cell Biol 103, 265a (1986); 15. Eur J Cell Biol 24, 176 (1981); 16. Proc Natl Acad Sci USA 74, 5613 (1977).

Related Products

Cat. No.	Product
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
29030... 29129	Streptavidin Conjugates
20203... 20502	Biotin Monoclonal Mouse Antibody (3D6.6)
22030	AntiFix™ Universal Antigen Retrieval Buffer, 10X
23001	EverBrite™ Mounting Medium
23002	EverBrite™ Mounting Medium with DAPI
23003	EverBrite™ Hardset Mounting Medium
23004	EverBrite™ Hardset Mounting Medium with DAPI
23016	EverBrite™ Hardset with NucSpot® 640
23008	Drop-n-Stain EverBrite™ Mounting Medium
23009	Drop-n-Stain EverBrite™ Mounting Medium with DAPI
23017	EverBrite TrueBlack® Hardset Mounting Medium
23018	EverBrite TrueBlack® Hardset with DAPI
23019	EverBrite TrueBlack® with NucSpot® 640
70062-70064	ViaFluor® Live Cell Microtubule Stains
40060	RedDot™1 Far-Red Nuclear Stain, 200X in Water
40061	RedDot™2 Far-Red Nuclear Stain, 200X in DMSO
40083... 41040	NucSpot® Nuclear Stains
23005	CoverGrip™ Coverslip Sealant
23023-23024	Super^{HT} PAP Pen 2.0
22015	Fixation Buffer
22016	Permeabilization Buffer
22017	Permeabilization and Blocking Buffer
23007	TrueBlack® Lipofuscin Autofluorescence Quencher
23014	TrueBlack® Plus Lipofuscin Autofluorescence Quencher
22014	Bovine Serum Albumin 30% Solution
22002	Tween® 20

Please visit our website at www.biotium.com for information on our full selection of fluorescent CF® Dye conjugates, including labeled primary and secondary antibodies, streptavidin, Annexin V, α-bungarotoxin, lectins, dextrans, and cholera toxin subunit B. Biotium also offers Mix-n-Stain™ Antibody Labeling kits to covalently label antibodies, nanobodies, and small ligands with one of our CF® Dyes.