Revised: January 13, 2017

Product Information

Phalloidin Conjugates

CF™ Dye Phalloidin Conjugates

Catalog #	Unit Size	Conjugate	Abs/Em (nm)
00049-T	50 U	Phalloidin, CF™350	347/448
00049	300 U		
00034-T	50 U	Phalloidin, CF™405M	408/452
00034	300 U		
00054-T	50 U	Phalloidin, CF™430	426/498
00054	300 U		
00055-T	50 U	Phalloidin, CF™440	440/515
00055	300 U		
00042-T	50 U	Phalloidin, CF™488A	490/515
00042	300 U		
00051-T	50 U	Phalloidin, CF™532	527/568
00051	300 U		
00043-T	50 U	Phalloidin, CF™543	541/560
00043	300 U		
00040-T	50 U	Phalloidin, CF™555	555/565
00040	300 U		
00044-T	50 U	Phalloidin, CF™568	562/583
00044	300 U		
00045-T	50 U	Phalloidin, CF™594	593/614
00045	300 U		
00046-T	50 U	Phalloidin, CF™633	630/650
00046	300 U		
00050-T	50 U	Phalloidin, CF™640R	642/662
00050	300 U		
00041-T	50 U	Phalloidin, CF™647	650/665
00041	300 U		
00052-T	50 U	Phalloidin, CF™660C	667/685
00052	300 U		
00047-T	50 U	Phalloidin, CF™660R	663/682
00047	300 U		
00048-T	50 U	Phalloidin, CF™680	681/698
00048	300 U		

Other Phalloidin Conjugates

Catalog #	Unit Size	Conjugate	Abs (nm)
00028	100 U	Phalloidin, Biotin-XX	N/A
00030	300 U	Phalloidin, Fluorescein	496/516
00032	300 U	Phalloidin, Rhodamine 110	502/524
00027	300 U	Phalloidin, Rhodamine	540/565
00033	300 U	Phalloidin, Sulforhodamine 101 (Texas Red®)	591/608

One unit of fluorescent phalloidin is defined as the amount of material used to stain one sample of fixed cells in a 200 uL volume (see protocols below).

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 in methanol or water, stock solutions are stable for at least one year when stored -20°C, protected from light. If using water as the solvent, freeze in aliquots. While the small amount of toxin in a vial is not likely to pose a heath hazard, it should be handled with care using universal laboratory safety precautions.

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 protocol (next page).

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.

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. 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 nonmuscle 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 (3, 4). Phallotoxins also stabilize F-actin, inhibiting depolymerization by cytochalasins, potassium iodide and elevated temperatures. Because the phalloidin conjugates are small, with an approximate diameter of 12–15 Å 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 (5, 6). Fluorescent phalloidin can also be used to quantify the amount of F-actin in cells (7, 8).

Protocols

Preparation of Stock Solutions

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. For fluorescent phalloidins one unit is equivalent to 5 uL of 200 U/mL stock solution in a total staining volume of 200 uL. For biotin-XX-phalloidin, one unit is equivalent to 10 uL of 100 U/mL stock solution in a total staining volume of 200 uL.

Continued next page

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.

1. Wash cells 3 times with PBS.
2. Fix cells on ice with 3.75% 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 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 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.
7. Place the staining solution on the coverslip for 20 minutes at room temperature. To avoid evaporation, keep the coverslips inside a covered container and the chamber slides covered during the incubation.

   Note: Phalloidin conjugates also can be included with fluorescently-labeled antibodies in blocking buffer during the secondary antibody incubation step in your regular immunofluorescence staining protocol.
8. Wash 2-3 times with PBS.
9. For biotin phalloidin, continue with biotin detection using labeled streptavidin or anti-biotin antibody. For fluorescent phalloidins, proceed to imaging.
10. CF™ dye phalloidins are photostable enough to image in PBS, but for best results we recommend mounting with fluorescence antifade mounting medium.

    Note: 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.

    Note: Fluorescent dyes can affect the stability of phalloidin staining. For best results, store phalloidin-stained samples at 4°C, protected from light, and image within 24 hours, especially with CF™405M, CF™647, and CF™680 phalloidins. Staining with phalloidins conjugated to rhodamine-based CF™ dyes (CF™488A, CF™532, CF™546, CF™568, CF™594, CF™633, CF™640R and CF™660R) is stable for up to a week after staining when specimens are stored at 4°C, protected from light.

Staining Living Cells

Fluorescently-labeled phalloidin is not cell-permeant and have therefore has not been used extensively with living cells. However, living cells have been labeled by pinocytosis or unknown mechanism (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. Wieland, T. in Phallotoxins, Springer-Verlag, New York (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.#	Product Name
40061	RedDot™2 Far Red Nuclear Counterstain, 200X in DMSO
23001	EverBrite™ Mounting Medium
23002	EverBrite™ Mounting Medium with DAPI
23003	EverBrite™ Hardset Mounting Medium
23004	EverBrite™ Hardset Mounting Medium with DAPI
23005	CoverGrip™ Coverslip Sealant
22005	Mini Super^{HT} Pap Pen 2.5 mm tip, ~400 uses
22006	Super^{HT} Pap Pen 4 mm tip, ~800 uses
22015	Fixation Buffer
22016	Permeabilization Buffer
22017	Permeabilization and Blocking Buffer
22010	10% Fish Gelatin Blocking Buffer
23007	TrueBlack Lipofuscin Autofluorescence Quencher, 20X in DMF
22014	30% Bovine Serum Albumin Solution
22002	Tween®-20

Please visit **www.biotium.com** to view our full selection of CF™ dye and R-PE conjugates, including labeled primary and secondary antibodies, streptavidin, Annexin V, α-bungarotoxin, and Mix-n-Stain antibody labeling kits. Biotium also offers a variety of apoptosis and cell viability assays for flow cytometry analysis, including mitochondrial membrane potential dyes and NucView™488 Caspase-3 Substrate for live cells.

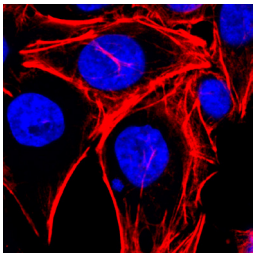

Figure 1. HeLa cells were fixed, permeabilized and stained with phalloidin, CF640R conjugate (red) and DAPI (blue).

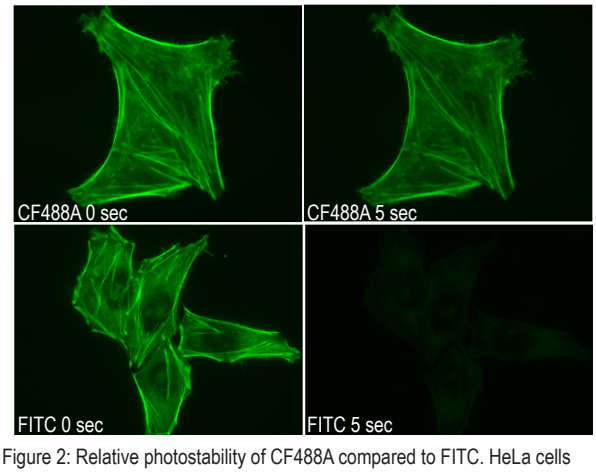

Figure 2: Relative photostability of CF488A compared to FITC. HeLa cells were stained with CF488A or FITC phalloidin conjugates and continuously exposed under a 100X objective using a mercury arc lamp microscope. Images were captured at t=0 and t=5 seconds of photobleaching.