Biotium
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Product Information

CF™ Dye Phalloidin Conjugates

Catalog #	Unit Size	Conjugate
00049-T	50 U	Phalloidin, CF™350
00049	300 U	
00034-T	50 U	Phalloidin, CF™405
00034	300 U	
00042-T	50 U	Phalloidin, CF™488A
00042	300 U	
00051-T	50 U	Phalloidin, CF™532
00051	300 U	
00043-T	50 U	Phalloidin, CF™543
00043	300 U	
00040-T	50 U	Phalloidin, CF™555
00040	300 U	
00044-T	50 U	Phalloidin, CF™568
00044	300 U	
00045-T	50 U	Phalloidin, CF™594
00045	300 U	
00046-T	50 U	Phalloidin, CF™633
00046	300 U	
00050-T	50 U	Phalloidin, CF™640R
00050	300 U	
00041-T	50 U	Phalloidin, CF™647
00041	300 U	
00047-T	50 U	Phalloidin, CF™660
00047	300 U	
00048-T	50 U	Phalloidin, CF™680R
00048	300 U	
00053-T	50 U	Phalloidin, CF™680
00053	300 U	

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.

Spectral Properties

Conjugate	Abs_{max} (nm)	Em_{max} (nm)
Phalloidin, CF™350	347	448
Phalloidin, CF™405	408	452
Phalloidin, CF™488A	490	515
Phalloidin, CF™532	527	558
Phalloidin, CF™543	541	560
Phalloidin, CF™555	555	565
Phalloidin, CF™568	562	583
Phalloidin, CF™594	593	614
Phalloidin, CF™633	630	650
Phalloidin, CF™640R	642	662
Phalloidin, CF™647	650	665
Phalloidin, CF™660	667	685
Phalloidin, CF™680	681	698
Phalloidin, CF™680R	680	701

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.

These phalloidins are labeled with our CF™ dyes, 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 Solution

Fluorescent phalloidin is supplied as a lyophilized solid. The material should be dissolved 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. One unit (U) of fluorescent phalloidin is defined as the amount of material used to stain one microscope slide of fixed cells, according to the following protocol (see below), and is equivalent to 5 uL of 200 U/

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mL stock solution in a total staining volume of 200 uL.

Staining Fixed Cells

The following protocol describes the staining procedure for adherent cells grown on glass coverslips or 8-well chamber slides.

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.

1. Wash cells 3 times with PBS.

2. Fix cells on ice with 3.75% formaldehyde solution in PBS for 15 minutes.

3. Wash 3 times with PBS.

4. Permeabilize cells with 0.5% Triton X-100 in PBS at room temperature for 10 min.

5. Wash cells 3 times with PBS.

6. Block non-specific binding using 5% BSA or 5% normal goat serum in PBS for 30 minutes.

7. Wash cells once with PBS.

8. Dilute 5 uL phalloidin stock solution in 200 uL PBS with 1% BSA for each cover slip or chamber to be stained.

9. 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.

10. Wash 2-3 times with PBS.

11. Mount with fluorescence antifade mounting medium (see related products)

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).

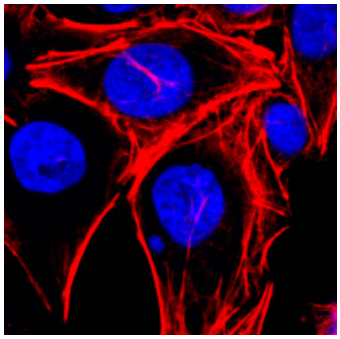

Figure 1. HeLa cells were fixed, permeabilized and stained with phalloidin, CF640R conjugate (red) and DAPI (blue).

Related Products

Cat.#	Product Name	Unit Size
40061-T	RedDot™2 Far Red Nuclear Counterstain, 200X in DMSO, Trial Size (15-20 tests)	25 uL
23001	EverBrite™ Mounting Medium	10 mL
23002	EverBrite™ Mounting Medium with DAPI	10 mL
23003	EverBrite™ Hardset Mounting Medium	10 mL
23004	EverBrite™ Hardset Mounting Medium with DAPI	10 mL
23005	CoverGrip™ Coverslip Sealant	15 mL
22005	Mini Super^{HT} Pap Pen 2.5 mm tip, ~400 uses	1 pen
22006	Super^{HT} Pap Pen 4 mm tip, ~800 uses	1 pen
22015	Fixation Buffer	100 mL
22016	Permeabilization Buffer	100 mL
22017	Permeabilization and Blocking Buffer	100 mL
22010	10% Fish Gelatin Blocking Buffer	100 mL
22011	Fish Gelatin Powder	2 x 50 g
22014	30% Bovine Serum Albumin Solution	100 mL
22002	Tween®-20	50 mL

Please visit **www.biotium.com** to view our full selection of CF™ dye and R-PE conjugates, including secondary antibodies, phalloidin, Annexin V, and α-bungarotoxin, as well as 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.

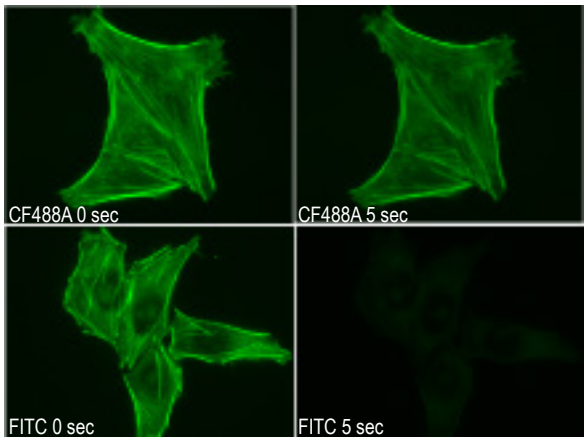

Figure 2: Relative photostability of CF488A compared to FITC. HeLa cells were stained with CF488A or FITC phalloidin conjugates and continuously exposed under a 100X objective using a mercury arc lamp microscope. Images were captured at t=0 and t=5 seconds of photobleaching.