

# Product Information

## ActinBrite™ High Affinity Phalloidin Conjugates

### Product List

Cat. No.	Unit Size*	Product
00095-T	50 U	ActinBrite™ 488/505 High Affinity Phalloidin Conjugate
00095	300 U	
00096-T	50 U	ActinBrite™ 530/555 High Affinity Phalloidin Conjugate
00096	300 U	
00097-T	50 U	ActinBrite™ 550/565 High Affinity Phalloidin Conjugate
00097	300 U	
00098-T	50 U	ActinBrite™ 610/630 High Affinity Phalloidin Conjugate
00098	300 U	
00101-T	50 U	ActinBrite™ 645/665 High Affinity Phalloidin Conjugate
00101	300 U	
00099-T	50 U	ActinBrite™ 665/690 High Affinity Phalloidin Conjugate
00099	300 U	
00100-T	50 U	ActinBrite™ 750/770 High Affinity Phalloidin Conjugate
00100	300 U	

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

### Storage and Handling

Store at -20°C, protected from light. Lyophilized product is stable for at least 12 months from date of receipt when stored as recommended.

To reconstitute ActinBrite™, add 1.5 mL of DMSO per vial for the 300 U size, or 0.25 mL of DMSO per vial for the 50 U size, and vortex to dissolve. This yields a stock solution of 200 U/mL, which is a 40X stock. After reconstitution, stock solutions are stable for at least 12 months when stored at -20°C, protected from light.

### Spectral Properties

Product	Excitation Laser Line(s) (nm)	Detection Channel
ActinBrite™ 488/505	488	FITC
ActinBrite™ 530/555	512, 532	Alexa Fluor® 532
ActinBrite™ 550/565	555, 561	Rhodamine
ActinBrite™ 610/630	594	Texas Red®
ActinBrite™ 645/665	633 - 640	Cy®5
ActinBrite™ 665/690	640	Alexa Fluor® 660
ActinBrite™ 750/770	730	Alexa Fluor® 750

### Product Description

Phalloidin is a toxin isolated from the deadly *Amanita phalloides* mushroom. It is a bicyclic peptide that binds specifically to F-actin. Fluorescently-labeled phalloidins are widely used probes for staining F-actin in fixed cells and tissue sections, but dye conjugation to the phalloidin peptide alters the affinity of the toxin for F-actin. While still binding with relatively high affinity, phalloidin staining is less stable than staining with other types of probes, like labeled antibodies. The stability of staining varies between different dye conjugates, and in general, samples cannot be stored for more than a week without loss of phalloidin signal. In addition, commonly used fluorescence mounting media may accelerate the loss of phalloidin binding.

ActinBrite™ High Affinity Phalloidin Conjugates were designed to preserve high phalloidin affinity for F-actin, resulting in bright staining that can be imaged more than one month after staining with minimal loss of signal or specificity.

ActinBrite™ High Affinity Phalloidins are available in a wide selection of fluorescent colors from green to near-IR for multiplexing flexibility. The dyes are named for their excitation and emission maxima. See the Spectral Properties table for recommended channels for imaging.

### Considerations for Staining

- The following protocol describes the staining procedure for adherent cells grown on glass coverslips or chamber slides. ActinBrite™ Phalloidins can also be used to stain fixed frozen or paraffin tissue sections, as well as yeast and fungi.
- Phalloidin staining is not compatible with methanol or acetone fixation, because these fixatives disrupt actin filaments. Formaldehyde or paraformaldehyde fixation is required. Methanol-free paraformaldehyde fixative is recommended for best results, though we have successfully stained with phalloidin conjugates after fixation with methanol-stabilized formalin.
- Phalloidin staining is compatible with upstream or downstream immunofluorescence staining. We recommend performing ActinBrite™ Phalloidin staining after immunolabeling.
- ActinBrite™ 530/555 and ActinBrite™ 550/565 are stable in EverBrite™ Mounting Medium for at least two weeks, or up to one month with some loss of brightness. The other ActinBrite™ Phalloidins are stable for at least one month in EverBrite™ Mounting Medium with minimal loss of brightness. Staining for all ActinBrite™ Phalloidins is stable in PBS for at least two months with minimal loss of brightness.

## Experimental Protocol

### Materials required but not provided

- PBS
- Paraformaldehyde, 4% in PBS (Cat. No. 22023) or other methanol-free formaldehyde fixative
- Triton® X-100

### Staining of fixed cells

1. Wash cells 3 times with buffer. For adherent cells, we recommend using HBSS with  $\text{Ca}^{2+}/\text{Mg}^{2+}$  to maintain cell attachment and morphology.
2. Fix cells on ice with 4% paraformaldehyde solution in PBS for 15 minutes. Fixation time or temperature may be varied if required for your experiment.
3. Rinse cells 3 times with PBS.
4. Permeabilize cells with 0.1-0.5% Triton® X-100 in PBS at room temperature for 10 minutes. Permeabilization conditions may be varied as required for your experiment. Permeabilization may also be done in combination with blocking for immunofluorescence staining.
5. Rinse cells 3 times with PBS.
6. Dilute 5  $\mu\text{L}$  of 40X ActinBrite™ Phalloidin stock solution in 195  $\mu\text{L}$  of PBS for each sample to be stained. Volumes can be scaled as necessary depending on the size of the specimen or culture well. The final concentration is 5 U/mL.  
**Note:** For staining yeast or fungi, use a 5X ActinBrite™ Phalloidin solution by diluting 25  $\mu\text{L}$  of 40X ActinBrite™ Phalloidin stock solution in 175  $\mu\text{L}$  of PBS for each sample to be stained. The final concentration is 25 U/mL.
7. Place the staining solution on the cells for 10 minutes or longer at room temperature. To avoid evaporation, keep the coverslips inside a covered container and the chamber slides covered during the incubation.
8. Rinse samples 2-3 times with PBS.
9. Image the samples in PBS or fluorescence antifade mounting media. See Spectral Properties table for recommended channels for imaging.

## Related Products

Cat. No.	Product
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
90082	DMSO, Anhydrous
00027... 00064	Phalloidin Conjugates
40083... 41040	NucSpot® Nuclear Stains
30131- 30140	CytoLiner™ Fixed Cell Membrane Stains
70062- 70064	ViaFluor® Live Cell Microtubule Stains
70054	MitoView™ Green
70065, 70069	LipidSpot™ Lipid Droplet Stains
22030	AntiFix™ Universal Antigen Retrieval Buffer, 10X
23007, 23011	TrueBlack® Lipofuscin Autofluorescence Quencher
23014	TrueBlack® Plus Lipofuscin Autofluorescence Quencher, 40X in DMSO
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
23017	EverBrite TrueBlack® Hardset Mounting Medium
23018	EverBrite TrueBlack® Hardset with DAPI
2019	EverBrite TrueBlack® with NucSpot® 640
23008	Drop-n-Stain EverBrite™ Mounting Medium
23009	Drop-n-Stain EverBrite™ Mounting Medium with DAPI
23005	CoverGrip™ Coverslip Sealant
23023, 23024	Super <sup>HT</sup> PAP Pen 2.0

Please visit our website at [www.biotium.com](http://www.biotium.com) for information on our full selection of fluorescent CF® Dye conjugates and reactive dyes, membrane stains, Mix-n-Stain™ antibody labeling kits, fluorescent probes, and kits for cell biology research.

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