Phalloidin-FluoProbes®

A popular probe for F-actin detection, available with over 30 labels

Product name cat.number	**MW** (g·mol-1)	$\lambda_{exc}\backslash\lambda_{em}$. **max.** (nm)	**mol. abs.** ($M^{-1}cm^{-1}$)
Phalloidin FP-547012, 1mg	788.90	-	-
Phalloidin - FluoProbes® 350 FP-1J8670, 300 units*	1332.37	353 / 432	19 000
Phalloidin - FluoProbes® 390A FP-YE5160, 10nmol		390 / 479	24 000
Phalloidin - FluoProbes® 405 FP-CA9870, 300 units*	1465.47	400 / 423	32 000
Phalloidin - FluoProbes® 415 FP-BP1240, 300 units*	1246.37	418 / 465	39 000
Phalloidin - FluoProbes® 425A FP-YE5170, 10nmol		436 / 484	45 000
Phalloidin - FluoProbes® 488 FP-YE5180, 10nmol FP-YE5181, 20nmol		493 / 519	80 000
Phalloidin - FluoProbes® 490 FP-JO2830, 300 units*		491 / 515	73 000
Phalloidin-X5-FluoProbes® 495 (lc-FITC) FP-47548A, 300 units		495 / 520	70 000
Phalloidin-X5-FluoProbes® 505 FP-AZ0130, 300 units*	1257.39	505 / 530	85 000
Phalloidin - Rhodamine (TRITC) FP-475741, 300 units	1247.30	542 / 565	80 500
Phalloidin - FluoProbes® 532A FP-YE5190, 10nmol		532 / 553	115 000
Phalloidin - FluoProbes® 547 FP-AZ0330, 300 units	1452,68	557 / 574	150 000

FT-AZ0330

Product name cat.number	**MW** (g·mol-1)	$\lambda_{exc}\backslash\lambda_{em}$. **max.** (nm)	**mol. abs.** ($M^{-1}cm^{-1}$)
Phalloidin-FluoProbes® 547H FP-BZ9620, 300 units	1654.78	550 / 572	150 000
Phalloidin - FluoProbes® 550A FP-YE5200, 10nmol		554 / 576	120 000
Phalloidin - FluoProbes® 555 FP-1J6340, 300 units		547 / 572	100 000
Phalloidin - FluoProbes® 556 FP-BV4620, 300 units		548 / 573	100 000
Phalloidin - FluoProbes® 565A FP-YE5210, 10nmol		563 / 592	120 000
Phalloidin - FluoProbes® 590A FP-YE5220, 10nmol		580 / 599	120 000
Phalloidin - FluoProbes® 594 FP-DZ2360, 300 units	1864.07	586 / 616	92 000
Phalloidin - FluoProbes® 633A FP-YE5230, 10nmol		629 / 657	130 000
Phalloidin - FluoProbes® 635 FP-1K0450, 300 units	1428.75	647 / 671	200 000
Phalloidin - SR101 (Sulfo-Rhodamine) FP- 033991, 300 units	1489.75	583 / 603	95 000
Phalloidin - FluoProbes® 647 FP-BA0320 , 300 units	1434.65	652 / 673	250 000
Phalloidin - FluoProbes® 647H FP-BZ9630, 300 units		650 / 670	250 000
Phalloidin - FluoProbes® 647N FP-YE-5240, 10nmol		644 / 669	
Phalloidin--FluoProbes® 655A FP-YE5250, 10nmol		663 / 684	125 000
Phalloidin - FluoProbes® 681 FP-BC3520, 300 units		691 / 708	125 000
Phalloidin - FluoProbes® 682 FP-BG0480, 300 units		690 / 709	140 000
Phalloidin - Biotin 96693A, 50 units	1300	-	-

* One unit of phallotoxin is defined as the amount of material used to stain one microscope slide of fixed cells, according to the following protocol, and is equivalent to 5 µL of methanolic stock solution for the fluorescent phallotoxins, and 10µl for the biotin-phalloidin.

Other labels and derivatives ([Lys7], {Asp7], [Orn7]) available: see related products

Storage: +4°C or -20°C (long term), desiccate, protect from light especially in solution (M) .
When stored properly, phalloidin products are stable for at least 1 year.

Hazards : Toxic + , R26 , UN= 3172 pack group II. See below 'Handling and Storage'

Introduction

Phalloidin (*Bicyclic(Ala-DThr-Cys-cis-4-hydroxy-Pro-Ala-2-mercapto-Trp-4,5-dihydroxy-Leu)(S-3→6)*) and its derivatives (biotinylated, fluorescent) are used to various analysis techniques (microscopy, FCM...), to label, identify, quantitate and stabilize F-actin in fixed and permeabilized tissue sections, cell cultures or cell free experiments. Phalloidin bind to a site at which few actin-binding proteins bind. So most of the F-actin in cells is available for phalloidin labeling. These properties make phalloidin more attractive than actin specific antibodies for fluorescence microscopy, giving high contracts staining.

The **unlabeled phalloidin** may be used as controls in blocking F-actin staining or in promoting actin polymerization.
The **biotin-XX phalloidin** allows to visualize actin filaments by electron microscopy using standard enzyme-mediated avidin/streptavidin techniques.
The **fluorescent labeled phalloidins** allow direct visualization by fluorescence microscopy, and by other techniques (FCM, FLISA...). Applications include localization in cells, expression level quantitation, dynamics...

Directions for use

Handling and Storage

As Phalloidin is toxic (LD_{50} of = 2 mg/kg), it should be handled with extreme caution. For phalloidin derivates, although the amount of derivatized toxin present in a vial is very small. [r], and toxicity have not be investigated, they should also be handled with care.
Unlabeled and biotinylated phalloidins are readily soluble in aqueous buffers.
For Phalloidin-FluoProbes® '10nmol' vials, add 1 ml of methanol to to yield a final concentration of 300 units/ml (equal to 10 nmol/ml).
For Phalloidin-FluoProbes® '300U' vials, add 1.5 ml of methanol to to yield a final concentration of 200 units/ml (equal to 6.6 nmol/ml).
The diluted stock solution can be stored one year at -20°C, when desiccated and protected from moisture and light.
Other solvents may be used, as DMF or DMSO. Even low concentration may be achieved with warm water.

Fluorescent staining for adherent cells

1. Fix the cells in 4% paraformaldehyde/PBS overnight at +4°C, wash with PBS twice then permeabilize in 0.1% Triton X-100/PBS for 10 min, and block in PBS/2% BSA for 1 h at room temperature. Wash again.
Note: Alternatively, 10min fixation at RT can be performed and followed by fixation in acetone at –20°C.

2. Mix 5 µl of Phalloidin-FluoProbes® (6.6 nmol/ml) to 200 µl of PBS.
Note: Biotin-phalloidin should be used at higher concentrations (10µl in 200µl).
Note: One could pre-incubate the fixed cells with PBS with 1% BSA to reduce eventual significant background.

3. F-actin is labeled with diluted Phalloidin-FluoProbes® (0.16 nmol/ml final concentration in PBS) for 20min to 1h at room temperature (4°C and 37°C is also suitable). The samples are mounted in 50% glycerol/PBS with 100 mg/ml 1,4-Diazabicyclo[2.2.2]octane (DABCO). The cells are examined by fluorescence microscopy.

In case of Biotin-phalloidin use, perform a secondary detection with a labeled streptavidin.

Fluorescent staining with simultaneous staining

- **Cytoskeletal Buffer (CB)**

Suspend 18.14 g of the biological buffer PIPES (free base; 60 mM) in 1L of double-distilled water. Add 6.50 g of HEPES buffer (27 mM), 3.80 g of EGTA (10 mM), and 0.99 g of magnesium sulfate (heptahydrate; 4 mM). Stir and adjust the pH to 7.0 with concentrated sodium hydroxide. The PIPES buffer crystals will not completely dissolve until the buffer pH nears neutrality, but should then form a clear solution.

- **Phosphate Buffered Saline with Calcium and Magnesium (PBS)**

Dissolve 0.2 g of potassium chloride, 0.2 g of monobasic potassium phosphate, 8.0 grams of sodium chloride, and 1.74 g of dibasic sodium phosphate (heptahydrate) in 1L of double-distilled water. After dissolving these reagents, add 0.132 g of calcium chloride dihydrate and 0.10 g of magnesium chloride hexahydrate. Adjust the pH to 7.2 with concentrated sodium hydroxide. Addition of the divalent alkaline earth metals to the buffer solution is helpful to ensure that uncondensed chromatin remains intact and contained within the nucleus during

the staining procedure, dramatically decreasing background fluorescence levels when using DNA probes excited by ultraviolet irradiation (DAPI and Hoechst). This buffer should be used with cryosections.

- **Mixed Aldehyde and Detergent Fixative**

Dissolve 3 g of electron microscope grade paraformaldehyde powder in 100 ml of CB with heating, and then filter when the solution appears clear. After cooling and filtering, add 1.5 ml of 20% Triton X-100 (made in CB) and 100 µl of 50% glutaraldehyde. The final concentration of reagents should be 3% paraformaldehyde, 0.3% Triton X-100, and 0.05% glutaraldehyde. Mix the resulting solution well before use. This reagent should be made fresh daily.

- **Blocking Buffer**

10% normal goat serum (**NGS**) in PBS containing 0.05% Triton X-100 (add 2-3 mg sodium azide per 100 ml of blocking buffer to eliminate the growth of microorganisms). If secondary antibodies to a host other than goat are being used, prepare the Blocking Buffer with normal serum from that species.

- **PBS-Triton Wash Buffer**

For wash sequences immediately before blocking and once again before staining with nuclear dyes, use PBS containing 0.05% Triton X-100.

- **PBS-Triton Wash Buffer with Blocking Serum**

For wash sequences between the primary and secondary antibody incubations and immediately after the secondary antibody treatment, use PBS containing 0.05%Triton X-100 and 1% normal host (goat) serum.

- **Primary Antibody Cocktail**

Add the appropriate volume of concentrated primary antibody stock solution to Blocking Buffer diluted 50% with PBS-Triton Wash Buffer (to yield a final concentration 5% normal goat serum). Several primary antibodies from different hosts can be mixed into a cocktail. Adherent cells on coverslips should be treated with 100 µl of primary antibody cocktail.

- **Secondary Antibody/Phalloidin Cocktail**

Add the appropriate volume of secondary antibody conjugated to the selected fluorophore (for example, 8 µl of goat anti-mouse secondary IgG with FluoProbes 647H at 2 mg/ml) to 1 ml of Blocking Buffer diluted 50% with PBS-Triton Wash Buffer (to yield a final concentration 5% normal goat or other host serum). If the cells are to be simultaneously counterstained with phalloidin, add 20-40 µl of concentrated stock solution (6.6 nmol/ml) to 1 ml of the Blocking Buffer. As with the primary antibody mixture, adherent cells on coverslips should be treated with 100 µl of primary antibody cocktail.

- **Nuclear Dye**

Prepare fresh dilutions of the nuclear dye immediately prior to staining. When using DRAQ5, and the cyanine nuclear stains, background fluorescence can be significantly reduced (especially in the cytoplasm) by adding 10 mg of RNase to the Blocking Buffer. In this case, block the cells or tissues at 37° C, rather than at room temperature, in order to activate the enzyme.

- **Nuclear Dye Wash Buffer**

Hoechst dyes require Hanks Balanced Salt Solution (**Hanks BSS**), while DAPI, as well as the monomeric and dimeric cyanine nuclear stains, can be used with PBS.

- **Nuclear Counterstain Dilutions**

- **Hoechst (33342 and 33258)** - Dilute 5 µl of 10 mg/ml stock solution in 150 milliliters of Hanks BSS (treat for 30 mn).
- **DAPI** - Dilute 5 µl of 10 mg/ml stock solution in 150 ml of PBS diluted 50% with double-distilled water (treat for 5 minutes).
- **Monomeric and Dimeric Cyanine Dyes** - Dilute the concentrated stock solution as recommended by the manufacturer (1:20 to 1:1000) into PBS (treat for 5 to 30 mn).
- **DRAQ5** - Dilute the concentrated stock solution (usually 1 millimolar) as recommended by the manufacturer (1:20 to 1:1000) into PBS (treat for 5 to 30 mn).

Procedure

Aspirate the growth medium from a Petri dish containing healthy cells adhered to coverslips and replace with pre-warmed (37°C) CB buffer to remove medium and serum proteins (use 3 ml of buffer for 60 mm Petri dishes). Wash the cells twice for 5 minutes (each wash) with the pre-warmed CB buffer, and incubate the cells at 37°C during the wash cycles. Each Petri dish should be individually marked with the primary antibodies and

other stains used for the coverslips in that dish. Coverslips should remain with the original Petri dish for each step in the entire procedure.

Fix the cells by adding the appropriate volume of pre-warmed (37°C) mixed aldehyde fixer to each Petri dish and rapidly transfer the dishes to the incubator. Fix for 10 mn.

Remove the Petri dishes from the incubator and wash once with CB buffer for 5 mn and twice with PBS-Triton Wash Buffer (5 mn each wash) before blocking.

Remove the Petri dishes from the incubator and wash once with CB buffer for 5 mn and twice with PBS-Triton Wash Buffer (5 mn each wash) before blocking.

Remove the PBS-Triton Wash Buffer and block nonspecific secondary antibody binding sites with 10% normal host serum Blocking Buffer. Treat the adherent cells for 60 mns at room temperature with the Blocking Buffer and slowly rotate the Petri dishes as the cells are being blocked on an orbital shaker at 5-10 revolutions per minute.

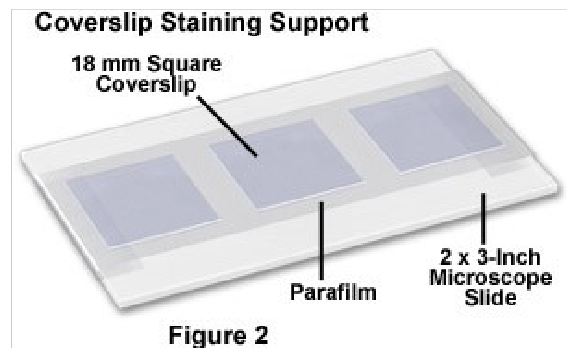

Figure 2

During the blocking step, prepare antibody treatment supports by covering 2 x 3” microscope slides with Parafilm, as illustrated in Figure 2. Secure the Parafilm so that it adheres tightly and is smoothly distributed along the glass surface (no blisters). After blocking, carefully remove the coverslips from the Petri dishes and place them cell-side down on a 100 µl drop of diluted primary antibody cocktail in 50% blocking buffer deposited on a Parafilm-covered slide. Between 3 and 6 coverslips (depending on size) can be placed on a single slide. Next, place the slides in a humidity chamber (see Figure 3) and incubate the coverslips in the humidity chamber for 1.5 hours at 37 degrees Celsius. If the primary antibodies are not conjugated to fluorophores, it is not necessary to protect the coverslips from light at this point.

After primary antibody treatment, return the coverslips to the original Petri dishes and wash three times at room temperature (5 to 10 mn for each wash) with PBS-Triton Wash Buffer with Blocking Serum to remove unbound primary antibodies. Slowly rotate the Petri dishes as the cells are being washed on an orbital shaker at 5-10 rpm.

After washing, carefully remove the coverslips from the Petri dishes and place them cell-side down on a 100 µl drop of diluted secondary antibody cocktail in 50% blocking buffer deposited on a Parafilm-covered slide. Once again, place the slides in a humidity chamber (see Figure 3) and incubate the coverslips in the humidity chamber for 1 hour at 37°C if smaller secondary antibody fragments are being used, or 1.5 h for entire antibody molecules. It is important to cover the humidity chamber with aluminum foil during this step to protect the fluorophores from light.

Figure 3

After secondary antibody treatment, return the coverslips to the original Petri dishes and wash three times at room temperature (5 to 10 mn for each wash) with PBS-Triton Wash Buffer with Blocking Serum to remove unbound secondary antibodies. Slowly rotate the Petri dishes as the cells are being washed on an orbital shaker at 5-10 rpm.

In preparation for nuclear staining, wash the cells twice with PBS-Triton Wash Buffer for 5 mn (each wash). Slowly rotate the Petri dishes as the cells are being washed on an orbital shaker at 5-10 rpm.

For DAPI and cyanine nuclear counterstains, add the diluted dye in PBS (dilute PBS to 50% with double-distilled water for DAPI) to the Petri dish and treat the coverslips for the recommended time: 5-10 mn for DAPI; 15-30 mn for cyanine dyes (protect from light with aluminum foil). When using Hoechst stains (30 mn

incubation), first wash the slides in Hanks Balanced Salt Solution for two buffer exchanges prior to counterstaining.

Wash the counterstained coverslips with either PBS or Hanks Balanced Salt Solution (depending upon the nuclear dye) for three times at 5 mn for each wash. Protect from light with aluminum foil.

In order to remove excess salt, wash the cells three times for 2 to 3 mn (each wash) in distilled water. Note that this step is only necessary if the coverslips are to be air-dried overnight before mounting.

After the final distilled water washing step, carefully remove the coverslips from the Petri dish with tweezers and wipe excess water from the back and edges. Lean the coverslips on their sides against the labeled Petri dish cover and allow them to dry overnight. Protect the drying coverslips from light with an aluminum baking tray. After drying, mount the coverslips (cell-side down) on clean microscope slides using the appropriate mounting medium.

Staining living cells [0]

Although Phallotoxins are usually not cell-permeant, some loading methods have been proposed: pinocytosis for hepatocytes, micro-injection for fibroblasts. Please search in the literature, and check with proper controls, as acting distribution and cell motility are usually affected.

Additional information

Phallotoxins (Phalloidin, and Phallacidin that differs by 2 aa), are bicyclic peptides isolated from the deadly *Amanita phalloides* mushroom. They bind competitively to the same sites in F-actin. Their unusual thioether bridge between a cysteine and tryptophan residue forms an inner ring structure. At elevated pH, this thioether is cleaved and the toxin loses its affinity for actin.
Phallotoxins have similar affinity for both large and small filaments, from many different species of plants and animals (unlike antibodies). Binding occurs in a stoichiometric ratio of about one phallotoxin molecule per actin subunit in muscle and non-muscle cells. Phallotoxins binding favors polymer formation, lowering the critical concentration for polymerization up to 30-fold. Phallotoxins also stabilize F-actin, inhibiting depolymerization by cytochalasins, potassium iodide, and elevated temperatures. Quantitation of F-actin is reported.

The Phalloidin binding do not affect actin function, and do not preclude the binding of other actin-binding proteins—including myosin, tropomyosin, troponin, and DNase I.

Beside conventional fluorescent labels (FITC, Rhodamine...), FluoProbes recommends the use of alternative labels that have the benefit of higher fluorescence and better photostability. Please consult FluoProbes for any advice.

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Other Phalloidin products

5-FAM-Phalloidin	JO4790	5-TAMRA-[Dap7]Phalloidin	JO4890
5-TAMRA-Phalloidin	JO4800	[Lys7]Phalloidin	JO4900
Coumarin-Phalloidin	JO4810	FITC-[Lys7]Phalloidin	JO4910
CR110-C6-Phalloidin	JO4820	CR110-C6-[Lys7]Phalloidin	JO4920
Biotin-C6-C6-Phalloidin	JO4830	5-FAM-[Lys7]Phalloidin	JO4930
Amino-Phalloidin	JO4840	5-TAMRA-[Lys7]Phalloidin	JO4940
[Dap7]Phalloidin	JO4850	TRITC-[Lys7]Phalloidin	JO4950
FITC-[Dap7]Phalloidin	JO4860	[Orn7]Phalloidin	JO4960
5-FAM-[Dap7]Phalloidin	JO4870	[Asp7]Phalloidin	JO4970
TRITC-[Dap7]Phalloidin	JO4880		

Related products

- DAPI, Pure Grade, FP-99963A
- Fluoro-Gel mounting medium, FP-AL2561
- Fluoro-Gel with DAPI & DABCO, FP-WYM680
- DABCO, 010558
- Phallacidin, control reagent, FP-JW6641
- FP547H Goat anti-mouse IgG (H+L), FP-SB4000
- FP647H Goat anti-mouse IgG (H+L), FP-SC4000

Ordering information

Catalog size quantities and prices may be found at http://www.fluoprobes.com.
Please inquire for higher quantities (availability, shipment conditions).
For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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