

Version 2 Last updated 23 May 2017

ab176762 CytoPainter Phalloidin-iFluor 750 Reagent

For staining actin filaments (F-actin) in formaldehyde-fixed cells and tissues.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

CytoPainter Phalloidin-iFluor 750 Reagent (ab176762) is one of a series of phalloidin conjugates that selectively binds to actin filaments, also known as F-actin. The iFluor 750 dye can be easily detected with a fluorescent microscope at Ex/Em = 752/778 nm (NIR range).

Our phalloidin conjugates are convenient probes for labeling, identifying and quantifying actin filaments in formaldehyde-fixed and permeabilized tissue sections, cell cultures or cell-free experiments. They can also be used in paraffin-embedded samples that have been de-paraffinized.

Phalloidin binds to actin filaments much more tightly than to actin monomers, leading to a decrease in the dissociation rate of actin subunits from filaments ends, essentially stabilizing actin filaments through the prevention of filament depolymerization.

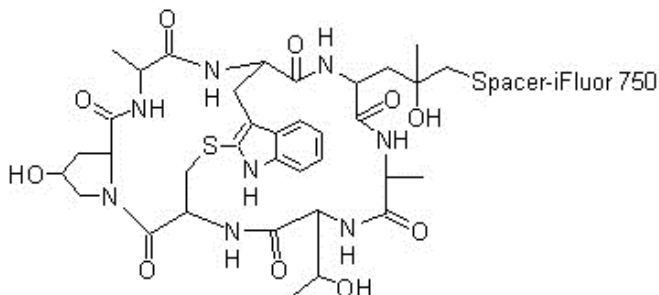
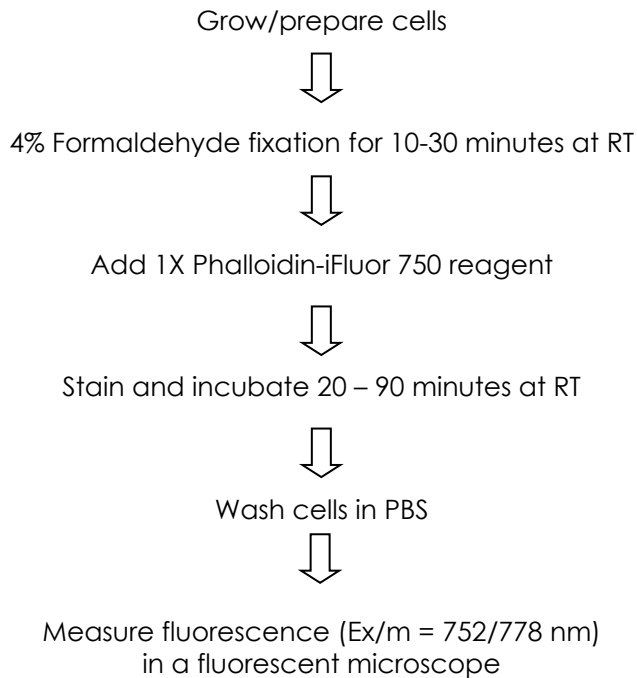


Figure 1. Chemical structure of Phalloidin-iFluor 750 Conjugate.

Actin is a globular, roughly 42-kDa protein found in almost all eukaryotic cells. It is also one of the most highly conserved proteins, differing by no more than 20% in species as diverse as algae and humans. Actin is the monomeric subunit of two types of filaments in cells: microfilaments, one of the three major components of the cytoskeleton, and thin filaments, part of the contractile apparatus in muscle cells. Thus, actin participates in many important cellular processes including muscle contraction, cell motility, cell division and cytokinesis, vesicle and organelle movement, cell signaling, as well as the establishment and maintenance of cell junctions and cell shape.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store reagent at -20°C in the dark immediately upon receipt.
Reagent has a storage time of 6 months from receipt.

5. Limitations

- Reagent intended for research use only. Not for use in diagnostic procedures.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Phalloidin-iFluor 750 Conjugate	300 tests	-20°C	-20°C

7. Materials Required, Not Supplied

These materials are not included, but will be required to successfully perform this assay:

- Fluorescence microscope fitted with a filter capable of detecting fluorescence at Ex/Em = 752/778 nm
- DMSO
- PBS
- PBS + 1% BSA
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- General tissue culture supplies
- Sterile 96 well plate with clear flat bottom, preferably black (if performing assay in microplate format). Use a poly-D-lysine coated plate for suspension cells
- 3-4% formaldehyde solution in PBS – for fixation step
- (Optional) Triton X-100: to add to PBS to increase permeability
- (Optional) DNA labeling reagent with different excitation/emission spectra to phalloidin-iFluor conjugate
- (Optional) Mounting media – we recommend Fluoroshield Mounting Media (ab104135)

8. Technical Hints

- This product is sold based on number of tests. A “test” simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this product meets your requirements. Please contact our Technical Support staff with any questions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vial at low speed prior to opening.

9.1 Phalloidin-iFluor 750 conjugate:

Prepare a 1000X Stock solution by dissolving the powder in the vial into 30 μ L of DMSO. Aliquot 1000X stock solution so that you have enough volume to perform the desired number of assays. Store at - 20°C protected from light. Avoid repeated freeze-thaw cycles.

Δ Note: Phalloidin is toxic. Although the amount of toxin present in the vial could be lethal only to a mosquito (LD₅₀ of phalloidin = 2 mg/kg), it should be handled with care.

10. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.
- The protocol described in this section has been optimized for staining in 96-well plate. Staining can also be performed in cover-slips inside a petri dish. In that case, you will need to modify the volume of the staining solution accordingly to the final volume.
- This protocol can be combined with an antibody-based staining. Phalloidin conjugate can be added either during the primary antibody incubation or during the secondary antibody / DNA staining incubation step.

Δ Note: The optimal concentration and incubation time of the Phalloidin-iFluor 750 conjugate will vary depending on the specific application. The staining conditions may be modified according to the particular cell type and/or the permeability of the cells or tissues to the probe.

10.1 Prepare 1X Phalloidin-iFluor 750 Working solution:

- 10.1.1 Add 1 μ L of the 1000X Phalloidin conjugate Stock solution in 1 mL of PBS + 1% BSA and mix well by pipetting up and down. This makes enough staining solution for 10 wells (100 μ L/well).

Δ Note: PBS without BSA can also be used to prepare working solution. Addition of BSA is preferred as it will minimize the chances of phalloidin sticking to the tube.

Δ Note: Do not store diluted 1X working solution. Simply make enough volume for the number of samples required.

- 10.1.2 Proceed to step 10.2 for adherent cell staining protocol or step 10.3 for suspension cell staining protocol.

10.2 Adherent cell staining:

- 10.2.1 Grow cells in a 96 well black wall/clear bottom plate with the appropriate culture medium till they reach desired confluence (recommendation: 70-80%).

Δ Note: cells can also be grown cover-slips inside a petri dish. In that case, you will need to modify the volume of the staining solution accordingly to the final volume.

10.2.2 Aspirate cell culture medium carefully to avoid dislodging any cells from the plate.

10.2.3 Wash once in PBS.

10.2.4 Formaldehyde fixation: incubate cells in 3-4% formaldehyde in PBS at room temperature for 10-30 minutes.

Δ Note: avoid any methanol containing fixatives since methanol can disrupt actin during the fixation process. The preferred fixative is methanol-free formaldehyde.

10.2.5 Aspirate staining solution carefully and wash fixed cells 2-3 times in PBS.

10.2.6 Optional: add 0.1% Triton X-100 in PBS into the fixed cells for 3-5 minutes to increase permeability. Wash permeabilized cells 2-3 times in PBS.

10.2.7 Add 100 μ L of 1X Phalloidin conjugate working solution (Step 10.1) to each well of fixed cells. Incubate cells at room temperature for 20-90 minutes.

Δ Note: if using, you can add DNA staining dye at this point.

10.2.8 Rinse cells gently 2-3 times with PBS to remove excess phalloidin conjugate.

10.2.9 Add mounting media (to preserve fluorescence) and seal (if using coverslips).

10.2.10 Observe the cells by using a fluorescence microscope fitted with appropriate filter at Ex/Em = 752/778 nm.

10.3 Suspension cell staining:

10.3.1 Grow cells in the appropriate culture vessel until they reach the desired confluence (70-80%).

Δ Note: Suspension cells may be attached to microplate or coverslips that have been treated with poly-D-lysine can be stained following the procedure for adherent cells (Step 10.2).

10.3.2 Centrifuge suspension cells at 1,000 rpm for 5 minutes to obtain a cell pellet and aspirate the supernatant.

10.3.3 Resuspend the cell pellets gently in pre-warmed (37°C) growth medium and transfer to microplate or coverslips.

10.3.4 Aspirate cell culture medium carefully to avoid dislodging any cells from the plate. Wash once in PBS.

10.3.5 Formaldehyde fixation: incubate cells in 3-4% formaldehyde in PBS at room temperature for 10-30 minutes.

Δ Note: avoid any methanol containing fixatives since methanol can disrupt actin during the fixation process. The preferred fixative is methanol-free formaldehyde.

10.3.6 Aspirate staining solution carefully and wash fixed cells 2-3 times in PBS.

10.3.7 Optional: add 0.1% Triton X-100 in PBS into the fixed cells for 3-5 minutes to increase permeability. Wash permeabilized cells 2-3 times in PBS.

10.3.8 Add 100 μ L of 1X Phalloidin conjugate working solution (Step 10.1) to each well of fixed cells. Incubate cells at room temperature for 20-90 minutes.

Δ Note: if using, you can add DNA staining dye at this point.

10.3.9 Rinse cells gently 2-3 times with PBS to remove excess phalloidin conjugate.

10.3.10 Add mounting media (to preserve fluorescence) and seal (if using coverslips).

10.3.11 Observe the cells by using a fluorescence microscope fitted with appropriate filter at Ex/Em = 752/778 nm.

11. Data Analysis

- We recommend acquiring several images per well.
- We recommend data analysis after coding and mixing images to ensure unbiased results.
- For manual analysis, if you do not have a specific software installed in your microscope, you can download ImageJ, an open source image processing designed for scientific multidimensional images by the National Institute of Health (NIH).

12. Typical data

Data provided for **demonstration purposes** only.

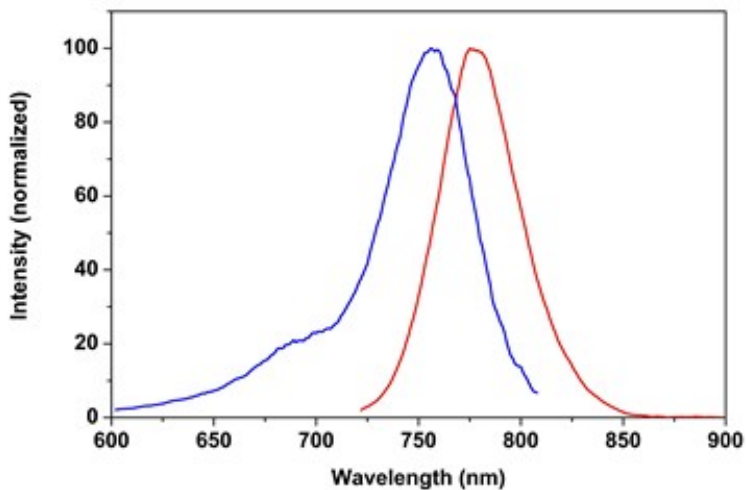


Figure 2. Excitation and emission spectra of CytoPainter Phalloidin-iFluor 750 reagent (ab176762).

13. Troubleshooting

Problem	Reason	Solution
Actin filaments not sufficiently stained	Low dye concentration / Incubation time insufficient	Increase dye concentration and/or incubation time
	Cells analysed at incorrect wavelength	Ensure you are using the appropriate filter settings
Cells do not appear healthy	Cells require serum to remain healthy	Add serum (2-10% range) to stain and wash solutions
Nuclear counterstain is too bright	Different microscopes, cameras and filters may make some signals appear very bright	Reduce concentration of nuclear counterstain or shorten exposure time

14. Notes

Technical Support

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Austria

wissenschaftlicherdienst@abcam.com | 019-288-259

France

supportscientifique@abcam.com | 01.46.94.62.96

Germany

wissenschaftlicherdienst@abcam.com | 030-896-779-154

Spain

soportecientifico@abcam.com | 91-114-65-60

Switzerland

technical@abcam.com

Deutsch: 043-501-64-24 | Français: 061-500-05-30

UK, EU and ROW

technical@abcam.com | +44(0)1223-696000

Canada

ca.technical@abcam.com | 877-749-8807

US and Latin America

us.technical@abcam.com | 888-772-2226

Asia Pacific

hk.technical@abcam.com | (852) 2603-6823

China

cn.technical@abcam.com | 400 921 0189 / +86 21 2070 0500

Japan

technical@abcam.co.jp | +81-(0)3-6231-0940

Singapore

sg.technical@abcam.com | 800 188-5244

Australia

au.technical@abcam.com | +61-(0)3-8652-1450

New Zealand

nz.technical@abcam.com | +64-(0)9-909-7829