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# ab176755 CytoPainter Phalloidin-iFluor 532 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.

# Table of Contents

# 1. Overview

CytoPainter Phalloidin-iFluor 532 Reagent (ab176755) is one of a series of phalloidin conjugates that selectively binds to actin filaments, also known as F-actin. The iFluor 532 dye can be easily detected with a fluorescent microscope at Ex/Em = 542/558 nm.

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.

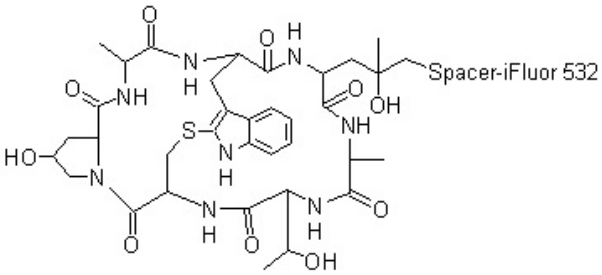
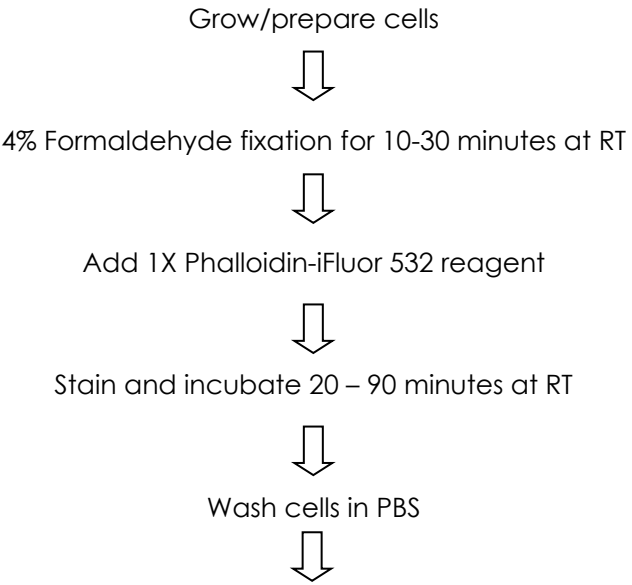



**Figure 1.** Chemical structure of Phalloidin-iFluor 532 Conjugate.

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 532 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 = 542/558 nm
- 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 532 conjugate (1000X stock):

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot 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 ($LD_{50}$ 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 532 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 532 Working solution:

10.1.1 Add 1 µL of the 1000X Phallodin 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 µL/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 µL 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 = 542/558 nm.

### 10.3 Suspension cell staining:

10.3.1 Grow cells in the appropriate culture vessel until the 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 µL 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 = 542/558 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.

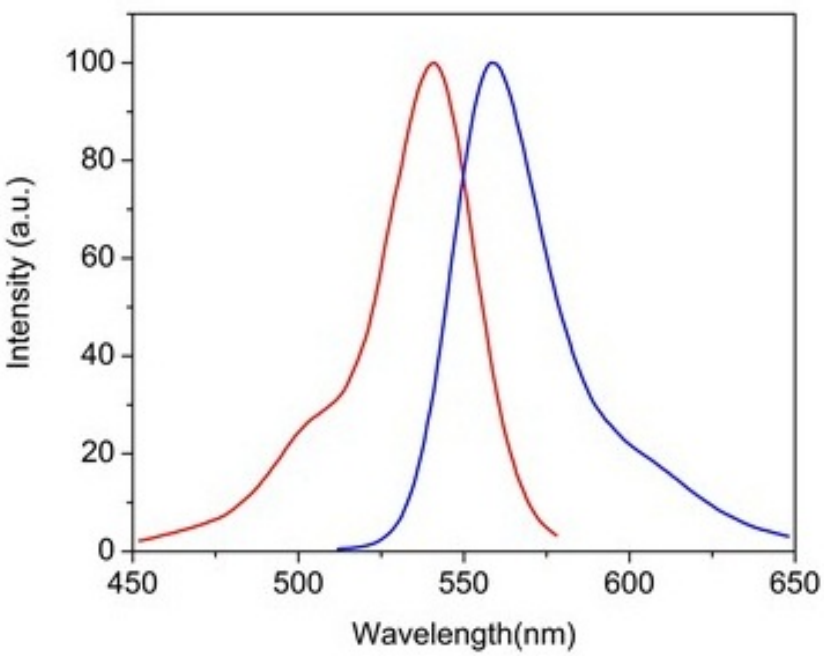



**Figure 2.** Excitation and emission spectra of CytoPainter Phalloidin-iFluor 532 reagent (ab176755).

## 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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