

ab176756 Phalloidin-iFluor 555 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.

For overview, typical data and additional information please visit: www.abcam.com/ab176756 (use abcam.cn/ab176756 for China, or abcam.co.jp/ab176756 for Japan)

Materials Supplied and Storage

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

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Phalloidin-iFluor 555 Conjugate	300 tests	-20°C	-20°C

Materials Required, Not Supplied

- Fluorescence microscope fitted with a filter capable of detecting fluorescence at Ex/Em = 556/574 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)

1. Reagent Preparation

Briefly centrifuge small vial at low speed prior to opening.

Phalloidin-iFluor 555 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 (LD₅₀ of phalloidin = 2 mg/kg), it should be handled with care.

2. 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 555 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.

2.1 Prepare 1X Phalloidin-iFluor 555 Working solution:

2.1.1 Add 1 μL 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 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.

2.1.2 Proceed to step 2.2 for adherent cell staining protocol or step 2.3 for suspension cell staining protocol.

2.2 Adherent cell staining:

2.2.1 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.

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

2.2.3 Wash once in PBS.

2.2.4 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.

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

2.2.6 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.

2.2.7 Add 100 μL of 1X Phalloidin conjugate working solution (Step 2.1) 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.

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

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

2.2.10 Observe the cells by using a fluorescence microscope fitted with appropriate filter at Ex/Em = 556/574 nm.

2.3 Suspension cell staining:

2.3.1 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 2.2).

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

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

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

2.3.5 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.

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

2.3.7 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.

2.3.8 Add 100 μL of 1X Phalloidin conjugate working solution (Step 2.1) 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.

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

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

2.3.11 Observe the cells by using a fluorescence microscope fitted with appropriate filter at Ex/Em = 556/574 nm.

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