ab176759 - CytoPainter Phalloidin-iFluor 647 Reagent

For staining actin filaments (F-actin) in formaldehyde-fixed cells and tissues.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab176759
(use www.abcam.cn/ab176759 for China, or www.abcam.co.jp/ab176759 for Japan)

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.
- 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.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website www.abcam.com.

Materials Supplied:

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Phalloidin-iFluor 647 Conjugate	300 tests	-20°C	-20°C

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

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 = 650/665 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)

Reagent Preparation

Before opening, briefly centrifuge the small vial at low speed to ensure all contents are collected at the bottom and to prevent loss of material.

Phalloidin-iFluor 647 conjugate 1000X Stock Solution:
Prepare a 1000X Stock solution by dissolving the powder in the vial into 30 µL 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.

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.

Prepare 1X Phalloidin-iFluor 647 conjugate Working solution:
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).

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

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.

Note: The optimal concentration and incubation time of the Phalloidin-iFluor 647 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.

Proceed to **step 1** for adherent cell staining protocol or **step 10** for suspension cell staining protocol.

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

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.

1. Aspirate cell culture medium carefully to avoid dislodging any cells from the plate.
2. Wash once in PBS.
3. Formaldehyde fixation: incubate cells in 3-4% formaldehyde in PBS at room temperature for 10-30 minutes.

Note: avoid any methanol containing fixatives since methanol can disrupt actin during the fixation process. The preferred fixative is methanol-free formaldehyde.
4. Aspirate staining solution carefully and wash fixed cells 2- 3 times in PBS.

5. 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.
6. 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.

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

7. Rinse cells gently 2-3 times with PBS to remove excess phalloidin conjugate.
8. Add mounting media (to preserve fluorescence) and seal (if using coverslips).
9. Observe the cells by using a fluorescence microscope fitted with appropriate filter at Ex/Em = 650/665 nm.

Suspension cell staining:

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

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

11. Centrifuge suspension cells at 1,000 rpm for 5 minutes to obtain a cell pellet and aspirate the supernatant.
12. Resuspend the cell pellets gently in pre-warmed (37°C) growth medium and transfer to microplate or coverslips.
13. Aspirate cell culture medium carefully to avoid dislodging any cells from the plate. Wash once in PBS.
14. Formaldehyde fixation: incubate cells in 3-4% formaldehyde in PBS at room temperature for 10-30 minutes.

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

15. Aspirate staining solution carefully and wash fixed cells 2- 3 times in PBS.
16. 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.
17. 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.

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

18. Rinse cells gently 2-3 times with PBS to remove excess phalloidin conjugate.
19. Add mounting media (to preserve fluorescence) and seal (if using coverslips).
20. Observe the cells by using a fluorescence microscope fitted with appropriate filter at Ex/Em = 650/665 nm.

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).

Trouble shooting

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

Technical Support

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