ab112124

CytoPainter F-actin Labeling Kit - Blue Fluorescence

Instructions for Use

For labeling sub-cellular organelles in tissue sections, cell cultures and cell free experiments using our proprietary blue fluorescence probe

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

Version: 4 Last Updated: 09 December 2013

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1. Introduction

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.

Abcam CytoPainter imaging kits are a set of fluorescence imaging tools for labeling sub-cellular organelles such as membranes, lysosomes, mitochondria, nuclei, etc. The selective labeling of live cell compartments provides a powerful method for studying cellular events in a spatial and temporal context.

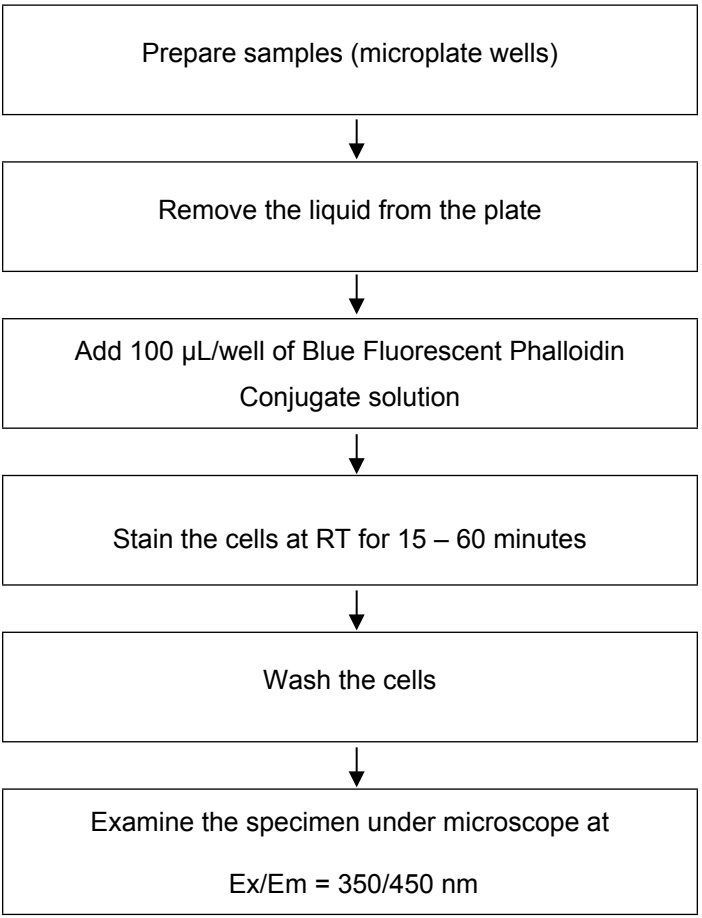
ab112124 is designed to label F-actins in fixed cells with blue fluorescence. The kit uses a blue fluorescent phalloidin conjugate that selectively binds to F-actins. When used at nanomolar concentrations, phallotoxins are convenient probes for labeling, identifying and quantitating F-actins in formaldehyde-fixed and permeabilized tissue sections, cell cultures or cell-free experiments.

ab112124 provides all the essential components with an optimized labeling protocol, which is robust requiring minimal hands on time.

The phalloidin conjugate has Ex/Em = 350/450 nm, compatible with DAPI filter set that comes with most of fluorescence microscopes.

2. Protocol Summary

Summary for One 96-well Plate



Note: Thaw all the kit components to room temperature before starting the experiment.

3. Kit Contents

Components	Amount
Component A: Blue Fluorescent Phalloidin Conjugate	1 x vial
Component B: Labeling Buffer	1 x 50 mL

4. Storage and Handling

Keep at -20°C. Avoid exposure to light.

5. Assay Protocol

Note: This protocol is for one 96 - well plate.

F-ACTIN STAINING ONLY

A. Preparation of 1X Blue Fluorescent Phalloidin Conjugate Solution

Add 10 µL Blue Fluorescent Phalloidin Conjugate (Component A) to 10 mL of Labeling Buffer (Component B).

Note 1: The unused Blue Fluorescent Phalloidin Conjugate stock solution (Component A) should be aliquoted and stored at -20 °C. Protect from light.

Note 2: Different cell types might be stained differently. The concentration of Blue Fluorescent Phalloidin Conjugate working solution should be prepared accordingly.

B. Staining the Cells

1. Perform formaldehyde fixation. Incubate the cells with 3.0 – 4.0 % 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

2. Rinse the fixed cells 2 – 3 times in PBS.

3. *Optional*: Add 0.1% Triton X-100 in PBS into fixed cells (from Step B.2) for 3 – 5 minutes to increase permeability. Rinse the cells 2 – 3 times in PBS.

4. Add 100 µL/well (96-well plate) of 1X Blue Fluorescent Phalloidin Conjugate working solution (from Step A) into the fixed cells (from Step B.2 or B.3), and stain the cells at room temperature for 15 – 60 minutes.

5. Rinse cells gently with PBS 2 – 3 times to remove excess dye before plate sealing and imaging by using DAPI channel.

ANTIBODY AND F-ACTIN COMBINATION STAINING

Note: This protocol is for one 96 - well plate.

A. Preparation of 1X Blue Fluorescent Phalloidin Conjugate Solution

Add 10 µL Blue Fluorescent Phalloidin Conjugate (Component A) to 10 mL of Labeling Buffer (Component B).

Note 1: The unused Blue Fluorescent Phalloidin Conjugate stock solution (Component A) should be aliquoted and stored protected from light at -20 °C.

Note 2: Different cell types might be stained differently. The concentration of Blue Fluorescent Phalloidin Conjugate working solution should be prepared accordingly.

B. Staining the Cells

1. Perform formaldehyde fixation. Incubate the cells with 3.0 – 4.0 % 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

2. Rinse the fixed cells 2 – 3 times in PBS.

3. Permeabilize fixed cells with 0.2% Triton X-100 in PBS; incubate for 10 – 30 minutes to increase permeability. Rinse the cells once with PBS.

4. Add 100 µl/well 5% Goat serum and leave for 30 minutes. Aspirate serum but do not rinse.

5. Add 100µl of primary antibody solution with 0.5% BSA in PBS and incubate for 1 hour. Make sure the entire are is covered.

6. Rinse cells 3 times in PBS, each time for 5 minutes. Aspirate PBS.

7. Add Goat serum, incubate for 1 – 2 minutes and aspirate.

8. Add 100 µL/well of secondary antibody solution diluted in 0.5% BSA in PBS buffer and 100 µL/well 1X Blue Fluorescent Phalloidin Conjugate per well (from Step A) working solution and stain the cells at room temperature for 30 – 60 minutes. Keep in the dark.

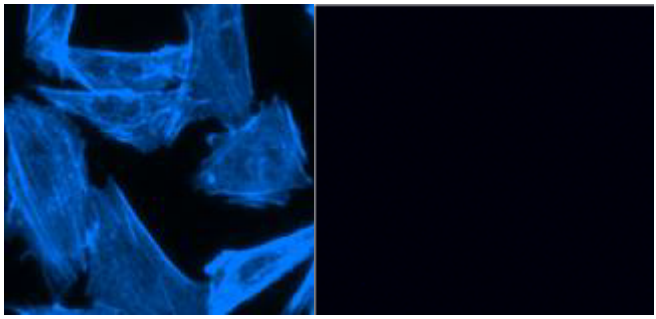
9. If desired, add nuclear dye at relevant dilution in 0.5% BSA in PBS and incubate 10 – 30 minutes. Wash cells 2 – 3 times for 5 minutes in PBS.

   Note: do not use DAPI as nuclear dye with this product as it emits in the same channel and the signal will be masked. We

recommend using DRAQ5 (ab108410) as it emits on the far red.

10. Seal plate and examine samples in fluorescence microscope using blue (DAPI) channel (Ex/Em = 350/450 nm) for F-actin staining. Use the appropriate channels to detect your secondary antibody and nuclear staining.

6. Data Analysis



A B

Figure 1. Images of CPA cells fixed with formaldehyde and stained with ab112124 in a black 96-well plate.

A: Cells were labeled with 1X Blue Fluorescent Phalloidin Conjugate for 30 minutes only.

B: Cells were pre-treated treated with phalloidin for 10 minutes, then stained with 1X Blue Fluorescent Phalloidin Conjugate for 30 minutes.

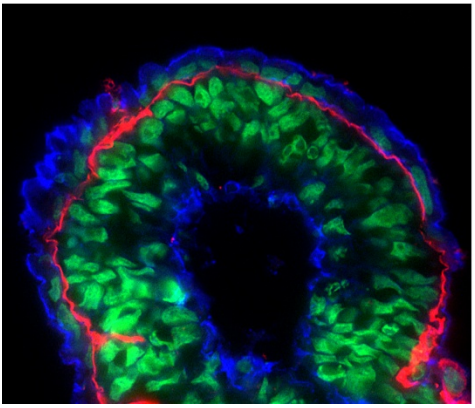


Figure 2. Mouse embryonic stem cell-differentiated embryonic bodies (EBs).

Red: Laminin alpha 1. Primary antibody: Rabbit anti-laminin alpha 1, 1:400. Secondary antibody: Goat polyclonal secondary antibody to rabbit IgG – H&L (Cy5®) (ab112124), 1:100

Blue: F-actin. CytoPainter F-actin staining kit – Blue fluorescence.

Green: nuclei. SYTO 16.

Image courtesy of Dr. Shaohua Li, UMDNJ-Robert Wood Johnson Medical School.

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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