Phalloidin-iFluor™ 488 Conjugate

Catalog number: 23115
Unit size: 300 Tests

Component	Storage	Amount
Phalloidin-iFluor™ 488 Conjugate	Freeze (< -15 °C), Minimize light exposure	300 Tests

OVERVIEW

This green fluorescent phalloidin conjugate (equivalent to Alexa Fluor® 488-labeled phalloidin) selectively binds to F-actins with much higher photostability than the fluorescein-phalloidin conjugates. Used at nanomolar concentrations, phalloidin derivatives are convenient probes for labeling, identifying and quantitating F-actins in formaldehyde-fixed and permeabilized tissue sections, cell cultures or cell-free experiments. Phalloidin binds to actin filaments much more tightly than to actin monomers, leading to a decrease in the rate constant for the dissociation of actin subunits from filament ends, essentially stabilizing actin filaments through the prevention of filament depolymerization. Moreover, phalloidin is found to inhibit the ATP hydrolysis activity of F-actin. Phalloidin functions differently at various concentrations in cells. When introduced into the cytoplasm at low concentrations, phalloidin recruits the less polymerized forms of cytoplasmic actin as well as filamin into stable "islands" of aggregated actin polymers, yet it does not interfere with stress fibers, i.e. thick bundles of microfilaments. The property of phalloidin is a useful tool for investigating the distribution of F-actin in cells by labeling phalloidin with fluorescent analogs and using them to stain actin filaments for light microscopy. Fluorescent derivatives of phalloidin have turned out to be enormously useful in localizing actin filaments in living or fixed cells as well as for visualizing individual actin filaments in vitro. Fluorescent phalloidin derivatives have been used as an important tool in the study of actin networks at high resolution. AAT Bioquest offers a variety of fluorescent phalloidin derivatives with different colors for multicolor imaging applications.

AT A GLANCE

Protocol Summary

1. Prepare samples in microplate wells
2. Remove liquid from samples in the plate
3. Add Phalloidin-iFluor™ 488 Conjugate solution (100 µL/well)
4. Stain the cells at room temperature for 20 to 90 minutes
5. Wash the cells
6. Examine the specimen under microscope with FITC filter

Important Warm the vial to room temperature and centrifuge briefly before opening.

Storage and Handling Conditions
The solution should be stable for at least 6 months if store at -20 °C. Protect the fluorescent conjugates from light, and avoid freeze/thaw cycles.

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

PREPARATION OF WORKING SOLUTION

Phalloidin-iFluor™ 488 Conjugate working solution
Add 1 µL of Phalloidin-iFluor™ 488 Conjugate solution to 1 mL of PBS with 1% BSA.

Note The stock solution of phalloidin conjugate should be aliquoted and stored at -20 °C. protected from light.

Note Different cell types might be stained differently. The concentration of phalloidin conjugate working solution should be prepared accordingly.

SAMPLE EXPERIMENTAL PROTOCOL

Stain the cells

1. Perform formaldehyde fixation. Incubate 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 for 3 to 5 minutes to increase permeability. Rinse the cells 2–3 times in PBS.

4. Add 100 µL/well (96-well plate) of Phalloidin-iFluor™ 488 Conjugate working solution into the fixed cells, and stain the cells at room temperature for 20 to 90 minutes.

5. Rinse cells gently with PBS 2 to 3 times to remove excess phalloidin conjugate before plating, sealing and imaging under microscope with FITC filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

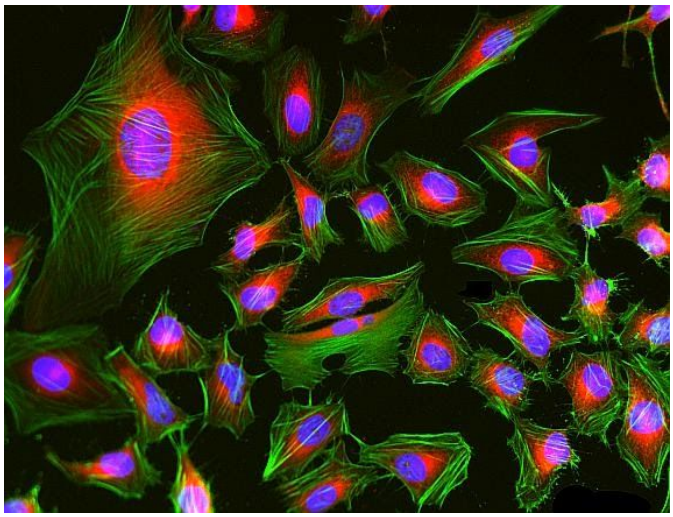

Figure 1.
Fluorescence images of HeLa cells stained with Phalloidin-iFluor™ 488 Conjugate using fluorescence microscope with a FITC filter set (Green). The cells were fixed in 4% formaldehyde, co-labeled with mitochondria dye MitoLite™ Red FX600 (Cat#2677, Red) and Nuclear Blue™ DCS1 (Cat#17548, Blue).

DISCLAIMER

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