

Phalloidin-iFluor® 514 Conjugate

Catalog number: 23116
Unit size: 300 Tests

Component	Storage	Amount (Cat No. 23116)
Phalloidin-iFluor™ 514 Conjugate	Freeze (< -15 °C), Minimize light exposure	300 Tests

OVERVIEW

This yellow fluorescent phalloidin conjugate (equivalent to Alexa Fluor® 514-labeled phalloidin) selectively binds to F-actins. Phalloidin derivatives are convenient probes used at nanomolar concentrations 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 and 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 valuable 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 been enormously helpful in localizing actin filaments in living or fixed cells and visualizing individual actin filaments in vitro. Fluorescent phalloidin derivatives have been used as an important tool in studying 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™ 514 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 TRITC filter

Important Note

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™ 514 Conjugate working solution

Add 1 µL of Phalloidin-iFluor™ 514 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™ 514 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 TRITC filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

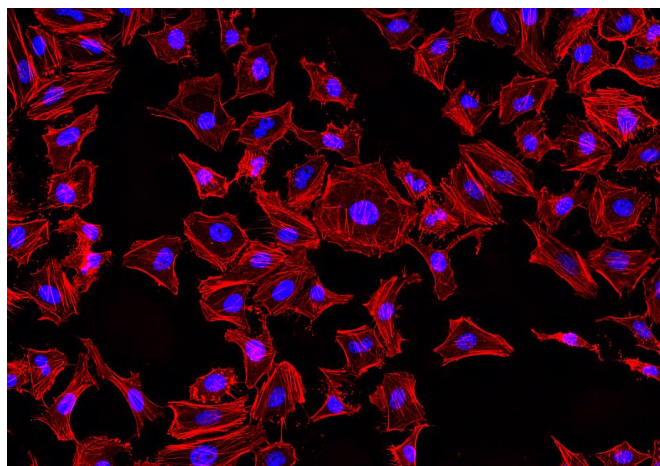


Figure 1. Fluorescence images of HeLa cells stained with Phalloidin-iFluor® 514 Conjugate using fluorescence microscope with a TRITC filter set (Red). Fixed cells were counterstained with Nuclear Blue™ DCS1 (Cat#17548, Blue).

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the

product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.