

## Phalloidin-iFluor® 488 Conjugate

Catalog number: 23115  
Unit size: 300 Tests

Component	Storage	Amount (Cat No. 23115)
Phalloidin-iFluor® 488 Conjugate	Freeze (< -15 °C), Minimize light exposure	300 Tests

### OVERVIEW

Phalloidin conjugates stain F-actin in fixed and permeabilized cells with high-affinity. Phalloidin is a bicyclic heptapeptide isolated from the poisonous death cap mushroom, \*Amanita phalloides\*. Its high binding affinity for the grooves between filamentous actin ([F-actin] (<https://www.aatbio.com/catalog/cell-structures-and-organelles-f-actin-filament>)) over monomeric G-actin is widely used to visualize and quantitate F-actin in tissue sections, cell cultures, or cell-free preparations. Compared to actin-specific antibodies, the non-specific binding of phalloidin is negligible, thus providing minimal background and high contrast during cellular imaging. Once bound to F-actin, phalloidin shifts the equilibrium of monomers and filaments toward the filaments side and inhibits ATP-hydrolysis. The interaction stabilizes actin filaments by preventing subunit dissociation, and it promotes actin polymerization by lowering the critical concentration. When conjugated to fluorescent dyes, phalloidin can be used at nanomolar concentrations to label and visualize F-actin in fixed and permeabilized cells, cell cultures, and cell-free experiments, as well as formaldehyde-fixed and permeabilized tissue sections. Phalloidin conjugates exhibit similar affinity for all types and sizes of actin filaments, binding in a stoichiometric ratio of 1:1 (phalloidin:actin) in both muscle and nonmuscle cells. Compared to antibodies, phalloidin derivatives are small (< 2 kDa), and phalloidin-bound filaments do not impede the functional properties of the filaments. The small size also permits denser F-actin labeling producing more detailed stains when imaged at higher resolutions. In addition, because actin is evolutionarily conserved, the binding properties of phalloidin derivatives can be utilized in staining a wide range of animal and plant cells.

### 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 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® 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

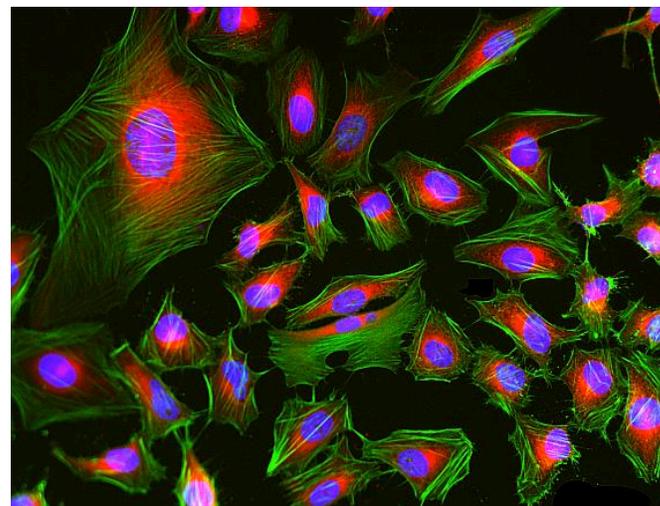
#### Stain the cells

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).

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