# Actin Lightning™ iFluor® 488 Phalloidin Conjugate

Catalog number: 22710
Unit size: 100 Tests

| Component | Storage | Amount (Cat No. 22710) |
|---|---|---|
| Actin Lightning™ iFluor® 488 Phalloidin Conjugate | Freeze (< -15 °C), Minimize light exposure | 100 Tests |

## OVERVIEW

Actin Lightning™ iFluor® 488 Phalloidin Conjugate is a novel fluorescent phalloidin conjugate designed for high affinity fluorescent staining of filamentous actin (F-actin). This high-affinity design supports bright and specific F-actin staining, allowing stained samples to be imaged for around one month after staining with minimal loss of signal or specificity under appropriate mounting and storage conditions. This conjugate is labeled with iFluor® 488, a green fluorophore with excitation and emission maxima of 491/516 nm. It is suitable for fluorescence microscopy workflows involving fixed cells and tissue sections, and provides a green channel option for imaging cell morphology, cytoskeletal organization, and F-actin distribution.

## AT A GLANCE

### Protocol Summary

1. Prepare samples in microplate wells
2. Remove liquid from samples in the plate
3. Add Actin Lightning™ iFluor® 488 Phalloidin Conjugate working solution (100 µL/well)
4. Stain the cells at room temperature for 20 to 90 minutes
5. Wash the cells and 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.

## KEY PARAMETERS

### Fluorescence microscope

| | |
|---|---|
| Emission | FITC filter set |
| Excitation | FITC filter set |
| Recommended plate | Black wall/clear bottom |

## PREPARATION OF WORKING SOLUTION

### Actin Lightning™ iFluor® 488 Phalloidin Conjugate Working Solution (1X):

Prepare working solution by adding 1 µL of Actin Lightning™ iFluor® 488 Phalloidin Conjugate stock solution into 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

This protocol only provides a guideline, and should be modified according to your specific needs.

### Cell Staining

1. Prepare cells in growth medium overnight.
2. Remove the cell culture medium and wash cells with HHBS or buffer of your choice.
3. 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.
4. Rinse the fixed cells 2–3 times in PBS.
5. **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.
6. Add 100 µL of Actin Lightning™ iFluor® 488 Phalloidin Conjugate working solution (1X).
7. Incubate cells in a cell incubator at room temperature for 20 to 90 minutes.
   **Note:** Incubating the dye for longer than 60 minutes can improve signal intensities in certain cell lines.
8. Remove the dye working solution and wash cells with PBS to remove any excess probes.
9. Image cells with fluorescence microscope using FITC filter set.

## EXAMPLE DATA ANALYSIS AND FIGURES

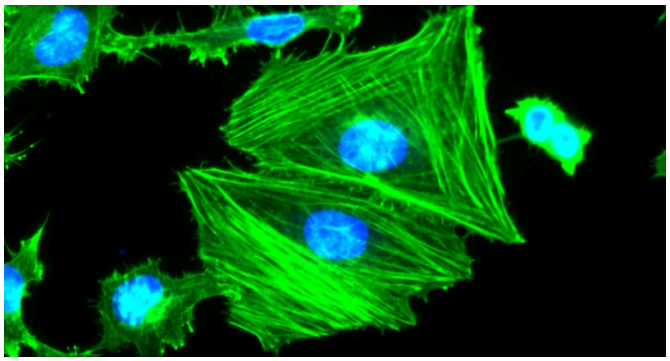

**Figure 1.** Fluorescence image of HeLa cells stained with Actin Lightning™ iFluor® 488 Phalloidin Conjugate and Nuclear Blue™ DCS1. Cells were fixed with 4% formaldehyde, permeabilized with 0.2% Triton™ X-100, then stained with Actin Lightning™ iFluor® 488 Phalloidin Conjugate (Cat. #22710) for F-actin detection and counterstained with Nuclear Blue™ DCS1 (Cat. #17548). Images were acquired using FITC and DAPI filter sets and overlaid to show F-actin in

green and nuclei in blue.

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