XFD488 Phalloidin

Catalog number: 23153
Unit size: 300 Tests

Component	Storage	Amount (Cat No. 23153)
AF488 Phalloidin [equivalent to Alexa Fluor® 488 phalloidin]	Freeze (< -15 °C), Minimize light exposure	300 Tests

OVERVIEW

XFD488 is manufactured by AAT Bioquest, and it has a chemical structure similar to that of Alexa Fluor® 488 (Alexa Fluor® is the trademark of Thermo Fisher). XFD488 phalloidin conjugate is chemically equivalent to Alexa Fluor® 488 phalloidin. This green fluorescent phalloidin conjugate selectively binds to F-actins. 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. 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 XFD488 Phalloidin 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.

KEY PARAMETERS

Fluorescence microscope

Emission	FITC filter
Excitation	FITC filter
Recommended plate	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

XFD488 Phalloidin Conjugate working solution

Add 1 µL of XFD488 Phalloidin 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 XFD488 Phalloidin 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

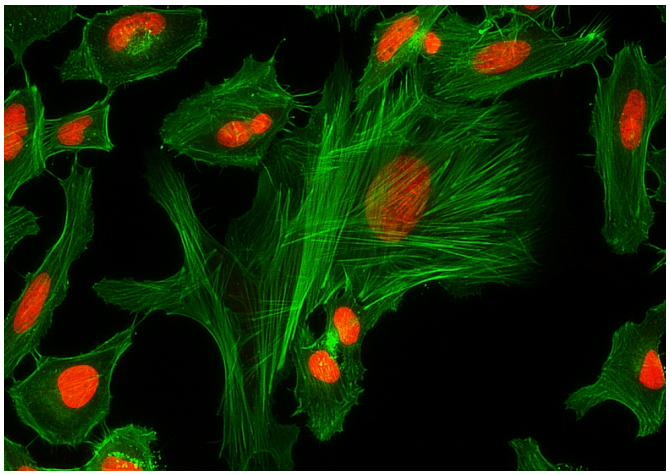

Figure 1. Fixed and stained HeLa cells. HeLa cells were fixed with 4% formaldehyde, permeabilized, and blocked. F-actin were stained with XFD488 phalloidin (Cat No. 23153) and nuclei labeled with Nuclear Red™ DCS1 (Cat No. 17552). Images were acquired on a Keyence BZ-X710 all-in-one fluorescence microscope.

DISCLAIMER

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