XFD647 Phalloidin

Catalog number: 23159
Unit size: 300 Tests

Component	Storage	Amount (Cat No. 23159)
XFD647 Phalloidin *equivalent to Alexa Fluor® 647 phalloidin*	Freeze (< -15 °C), Minimize light exposure	300 Tests

OVERVIEW

XFD647 phalloidin is a high-affinity probe for filamentous actin (F-actin), conjugated to XFD647, a bright, photostable, far-red fluorescent dye. XFD647, manufactured by AAT Bioquest, is structurally similar to Alexa Fluor™ 647 (Thermo Fisher), ensuring comparable performance in fluorescence applications. Phalloidin, a bicyclic peptide toxin derived from *Amanita phalloides* (commonly known as the death cap mushroom), is well known for its high specificity and strong binding affinity to F-actin. When conjugated to XFD647, phalloidin enables precise, high-contrast visualization of F-actin structures with minimal background interference. This fluorescent conjugate is highly effective for imaging F-actin in a wide range of biological specimens, including plant and animal tissues, fixed and permeabilized cells, and cell-free systems. XFD647 phalloidin binds to F-actin with nanomolar affinity, making it an excellent tool for labeling, identifying, and quantifying actin filaments. Its superior photostability and blinking properties make it particularly well suited for super-resolution microscopy techniques such as SIM and STORM. Additionally, XFD647 phalloidin staining is fully compatible with other fluorescent labels, including fluorescent proteins, Qdot nanocrystals, and iFluor®-conjugated secondary antibodies, allowing seamless integration into multiplex fluorescence imaging workflows.

AT A GLANCE

Protocol Summary

1. Prepare samples in microplate wells
2. Remove liquid from samples in the plate
3. Add XFD647 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 Cy3 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	Cy5 filter
Excitation	Cy5 filter
Recommended plate	Black wall/clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

XFD647 Phalloidin stock solution

1. Add 30 µL of DMSO to the powder and mix well.
2. Add 1 µL of the XFD647 Phalloidin 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 the phalloidin conjugate working solution should be prepared accordingly.

PREPARATION OF WORKING SOLUTION

XFD647 Phalloidin Conjugate working solution

1. Add 1 µL of XFD647 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 XFD647 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 Cy5 filter set.

DISCLAIMER

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