XFD555 Phalloidin *equivalent to Alexa Fluor® 555 phalloidin*

Catalog number: 23160
Unit size: 300 Tests

Component	Storage	Amount (Cat No. 23160)
XFD555 Phalloidin	Freeze (< -15 °C), Minimize light exposure	300 Tests

OVERVIEW

XFD555 phalloidin is a high-affinity F-actin probe conjugated to our bright, photostable, red-orange fluorescent XFD555 dye. XFD555, which is manufactured by AAT Bioquest, is structurally equivalent to Alexa Fluor™ 555 (ThermoFisher).

Phalloidin, a bicyclic peptide toxin derived from *Amanita phalloides* (commonly known as the death cap mushroom), is widely recognized for its high-affinity and selective binding to filamentous actin (F-actin). Fluorescently conjugated phalloidin exhibits exceptional specificity for F-actin across a diverse range of species, including both plant and animal systems, with minimal nonspecific interactions. The conjugation of phalloidin to XFD555, a highly photostable and intensely fluorescent red-orange dye, offers an optimal combination of target specificity and fluorescence performance. This conjugate enables high-contrast, low-background imaging, facilitating precise visualization and quantification of F-actin structures in various biological applications.

XFD555 phalloidin can be used to visualize and quantitate F-actin in tissue sections, cell cultures, or cell-free preparations. XFD555 phalloidin staining is fully compatible with other fluorescent stains used in cellular analyses including fluorescent proteins, Qdots, and other iFluor® conjugates including secondary antibodies.

AT A GLANCE

Protocol Summary

1. Prepare samples in microplate wells
2. Remove liquid from samples in the plate
3. Add XFD555 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	Cy3 filter
Excitation	Cy3 filter
Recommended plate	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

XFD555 Phalloidin Conjugate working solution

1. Add 1 µL of XFD555 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 XFD555 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 Cy3 filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

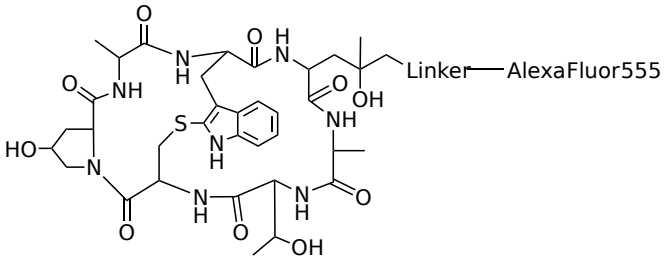

Figure 1. Chemical structure for XFD555 Phalloidin Conjugate.

DISCLAIMER

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