Phalloidin-iFluor® 660 Conjugate

Catalog number: 23126
Unit size: 300 Tests

Component	Storage	Amount (Cat No. 23126)
Phalloidin-iFluor® 660 Conjugate	Freeze (< -15 °C), Minimize light exposure	300 Tests

OVERVIEW

Phalloidin iFluor® 660 is an F-actin-specific probe conjugated to the far red-fluorescent dye iFluor® 660. Phalloidin, a bicyclic peptide derived from *Amanita phalloides* ("death cap" mushroom), is widely used for the selective labeling of filamentous actin (F-actin) in fluorescence microscopy.

The conjugation of phalloidin to iFluor® 660 retains its high binding affinity and specificity for F-actin, providing reliable and reproducible labeling across various biological systems. The iFluor® 660 fluorophore offers exceptional brightness and photostability, enabling prolonged imaging sessions with minimal photobleaching. Additionally, the probe exhibits negligible nonspecific binding, ensuring high-contrast visualization of actin filaments, even in complex biological environments.

Phalloidin iFluor® 660 is optimized for high-resolution imaging and quantitative analysis of F-actin in diverse applications, including fixed tissue sections, cultured cells, and in vitro actin polymerization assays. Its compatibility with multiplex fluorescence imaging allows co-labeling with fluorescent proteins, quantum dots, iFluor® derivatives, and antibody-based detection systems.

AT A GLANCE

Protocol Summary

1. Prepare samples in microplate wells.
2. Remove liquid from samples in the plate.
3. Add Phalloidin-iFluor® 660 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 Cy5 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 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

Phalloidin-iFluor® 660 Conjugate stock solution

1. Add 30 µL of DMSO into the powder and mix well.

PREPARATION OF WORKING SOLUTION

Phalloidin-iFluor® 660 Conjugate working solution

1. Add 1 µL of Phalloidin-iFluor® 660 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® 660 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.

EXAMPLE DATA ANALYSIS AND FIGURES

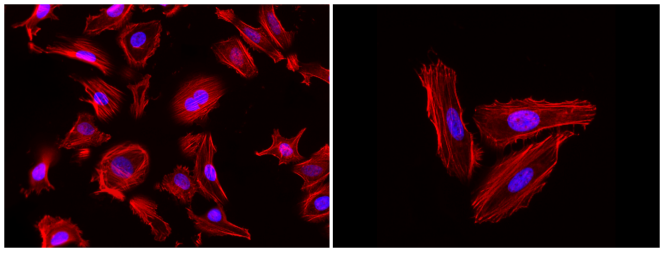

Figure 1. HeLa cells were fixed with 4% formaldehyde and subsequently stained with 0.25 μM Phalloidin-iFluor® 660 conjugate to label F-actin. Nuclei were counterstained with Hoechst 33342 (#17530) for 20 minutes at room temperature. Fluorescent imaging was performed using the Cy5 and DAPI channels, enabling precise visualization of F-actin localization and nuclear structures.

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