Phalloidin iFluor™ Conjugates

Dyes and Stains

Fluorescent dye conjugates for detection of F-actin in cell culture experiments

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Catalog #100-0852	300 Tests
Catalog #100-0853	300 Tests
Catalog #100-0854	300 Tests

Product Description

Phalloidin is a heptapeptide derived from the poisonous mushroom *Amanita phalloides*. Phalloidin binds to F-actin filaments in cells with high specificity, preventing filament depolymerization. Fluorescently labeled phalloidin can be used in labeling, identification, and quantitation of F-actin in formaldehyde-fixed and permeabilized cells. Fluorescently labeled phalloidin has lower non-specific binding compared to actin antibodies across most species of plants and animals, which provides low background and high contrast during imaging.

Product Information

PRODUCT NAME	CATALOG #	SIZE	STORAGE	SHELF LIFE
Phalloidin, iFluor™ 488	100-0852	300 Tests	Store at -20°C. Protect product from prolonged exposure to light.	Stable until expiry date (EXP) on box label.
Phalloidin, iFluor™ 555	100-0853	300 Tests	Store at -20°C. Protect product from prolonged exposure to light.	Stable until expiry date (EXP) on box label.
Phalloidin, iFluor™ 647	100-0854	300 Tests	Store at -20°C. Protect product from prolonged exposure to light.	Stable until expiry date (EXP) on box label.

Physical & Spectral Properties

PRODUCT NAME	FORMULATION	MOLECULAR WEIGHT (g/mol)	EXCITATION WAVELENGTH (nm)	EMISSION WAVELENGTH (nm)	EXTINCTION COEFFICIENT ($cm^{-1}M^{-1}$)**	QUANTUM YIELD**	CORRECTION FACTOR (280 nm)
Phalloidin, iFluor™ 488*	1000X solution in dimethyl sulfoxide (DMSO)	1399.49	491	516	75,000	0.9	0.11
Phalloidin, iFluor™ 555*		1468.53	557	570	100,000	0.64	0.14
Phalloidin, iFluor™ 647	Powder	1632.87	656	670	250,000	0.25	0.03

*Please refer to the Safety Data Sheet (SDS) for hazard information. This product contains components dissolved in DMSO. DMSO is a strong solvent and skin penetrant, and can transport many substances through the skin. DMSO can also penetrate some protective glove materials including latex and silicone. Extra caution should be utilized when handling this product.
**Measured with aqueous buffer (pH 7.2).

Directions for Use

Please read the entire protocol before proceeding. The following protocol is for staining cells in a black-wall/clear-bottom 96-well plate. If using other cultureware, adjust volumes accordingly. Staining may vary with cell type; the optimal concentration of working solution should be determined.

Preparation of Phalloidin iFluor™ Working Solution

1. Warm the Phalloidin iFluor™ vial to room temperature (15 - 25°C) and centrifuge briefly before opening.
2. Prepare a Phalloidin iFluor™ working solution as follows:
   - iFluor™ 488 and 555 conjugates: Add 1 µL of solution from the vial to 1 mL of phosphate-buffered saline (PBS) with 1% bovine serum albumin (BSA). Mix thoroughly. Use the working solution immediately; do not store.

     NOTE: If not used immediately, aliquot and store remaining solution from the vial at -20°C. Seal tubes tightly and protect from light. After thawing aliquots, use immediately; do not re-freeze.

- iFluor™ 647 conjugate:

a. To prepare a Phalloidin, iFluor™ 647 stock solution, add 30 µL of DMSO to the vial. Mix thoroughly.

   NOTE: If not used immediately, aliquot and store remaining solution from the vial at -20°C. Seal tubes tightly and protect from light. After thawing aliquots, use immediately; do not re-freeze.

b. To prepare a Phalloidin, iFluor™ 647 working solution, add 1 µL of stock solution from the vial to 1 mL of PBS with 1% BSA. Mix thoroughly. Use the working solution immediately; do not store.

Staining and Imaging Cells

1. Stain cells as follows:
   a. Plate cells in a black-wall/clear-bottom 96-well plate and treat cells as desired.
   b. Add 4% methanol-free formaldehyde in PBS to the cells, then incubate at room temperature for 10 - 30 minutes.

      NOTE: Avoid using a methanol-containing fixative, since methanol can disrupt actin during the fixation process.

   c. Remove fixative and rinse the fixed cells 2 - 3X with PBS.
   d. If desired, add 0.1% Triton™ X-100 in PBS to fixed cells and incubate at room temperature for 3 - 5 minutes to increase permeability. Rinse cells 2 - 3X with PBS.
   e. Add 100 µL/well of Phalloidin iFluor™ working solution to the fixed cells. Incubate at room temperature for 20 - 90 minutes; protect from light.

      NOTE: The optimal incubation time should be determined for different cell types.

   f. Rinse cells gently 2 - 3X with PBS to remove excess phalloidin conjugate.
2. Observe stained cells using a fluorescence microscope with the appropriate filter set, as follows:
   - iFluor™ 488 conjugate: FITC
   - iFluor™ 555 conjugate: Cy3/TRITC
   - iFluor™ 647 conjugate: Cy5