

**Product Information** 

# Phalloidin, Fluorescein Isothiocyanate Labeled

Sequence from *Amanita phalloides* (synthetic peptide sequence)

#### P5282

# **Product Description**

Molecular Formula:  $C_{56}H_{60}N_{10}O_{15}S_2$ 

Molecular Weight: 1177.26

Synonyms: Phalloidin-FITC, FITC-Phalloidin Molar Extinction Coefficient: 1 70,000 (495 nm)

Excitation wavelength: 495 nm Emission wavelength: 513 nm Storage temperature: -20 °C

Structure:

Phalloidin is a fungal toxin that occurs naturally in the poisonous mushroom *Amanita phalloides*. Phalloidin toxicity is attributed to the ability to bind F actin in liver and muscle cells. As a result of binding phalloidin, actin filaments become strongly stabilized. Phalloidin has been found to bind only to polymeric and oligomeric forms of actin, and not to monomeric actin. The dissociation constant of the actin-phalloidin complex has been determined to be on the order of 3  $\times$  10<sup>-8</sup> M.  $^4$ 

Fluorescent conjugates of phalloidin, such as FITC (fluorescein isothiocyanate) or TRITC (tetramethylrhodamine B isothiocyanate) conjugates, have been used to label actin filaments for histological applications,  $^{3-7}$  such as flow cytometry analysis of actin polymerization.  $^{8-10}$  Some structural features of phalloidin are required for the binding to actin.  $^3$  However, the side chain of amino acid 7 (y- $\delta$ -dihydroxyleucine) is accessible for chemical modifications without appreciable loss of affinity for actin. TRITC-phalloidin is considered to be less susceptible to photobleaching than FITC-phalloidin.  $^6$ 

#### Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

# Storage/Stability

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It is recommended to store this product at −20 °C.

# **Preparation Instructions**

This product is tested for solubility in methanol at 2 mg/mL. In general, solutions of phalloidin compounds should be prepared fresh and protected from light whenever possible. Several publications have reported preparation of stock solutions of FITC-phalloidin in organic solvents besides methanol, although we have not tested any of the following situations ourselves:

- 0.08% (0.8 mg/mL, or 800 µg/mL) stock solution of FITC-phalloidin in ethanol<sup>11</sup>
- 6.6 μM stock solution of FITC-phalloidin in ethanol<sup>12</sup>
- 0.1 mg/mL stock solution of FITC-phalloidin in DMSO<sup>13</sup>



## **Procedure**

The following procedure may serve as a general guideline for staining cells. <sup>14</sup> Final staining solutions in aqueous physiological buffers have a phalloidin concentration range of 0.1-100  $\mu$ M, with corresponding incubation times of 15 minutes to 72 hours.

- Cells are washed with phosphate buffered saline (PBS).
- 2. Cells are fixed for 5 minutes in 3.7% formaldehyde solution in PBS, then washed extensively in PBS.
- 3. Cells may be dehydrated with acetone, permeabilized with 0.1% TRITON™ X-100 in PBS and washed again in PBS.
- Cells are stained with a 50 μg/mL fluorescent phalloidin conjugate solution in PBS (containing 1% DMSO from the original stock solution) for 40 minutes at room temperature.
- 5. Wash several times with PBS to remove unbound phalloidin conjugate.

### References

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