Product Information

Phalloidin, Tetramethylrhodamine B isothiocyanate

Sequence from *Amanita phalloides* (synthetic peptide sequence)

P1951

Product Description

Molecular Formula: $C_{60}H_{70}N_{12}O_{15}S_2$

Molecular Weight: 1231.40

Synonyms: Phalloidin-TRITC, TRITC-Phalloidin, Rhodamine-Phalloidin, Rhodamine Phalloidin-TRITC

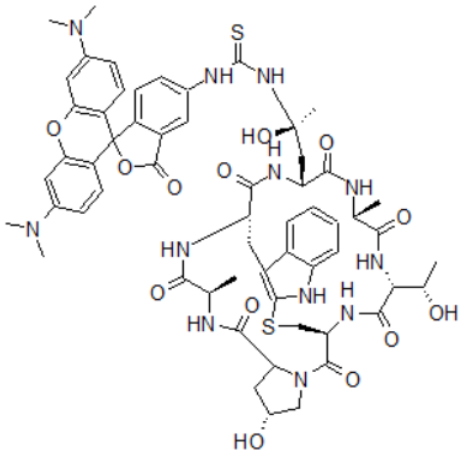
Molar Extinction Coefficient:[1] 80,000 (545 nm)

Excitation wavelength:[1] 540-545 nm

Emission wavelength:[1] 570-573 nm

Storage temperature: –20 °C

Structure:



Phalloidin is a fungal toxin that occurs naturally in the poisonous mushroom *Amanita phalloides*.[2] 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.[3] The dissociation constant of the actin-phalloidin complex has been determined to be on the order of 3×10^{-8} M.[4]

Fluorescent conjugates of phalloidin, such as FITC (fluorescein isothiocyanate) or TRITC (tetramethylrhodamine B isothiocyanate) conjugates, have been used to probe actin filaments for histological applications.[3-10] Some structural features of phalloidin are required for the binding to actin.[3] However, the side chain of amino acid 7 (γ-δ-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] The binding positions of phalloidin and of TRITC-phalloidin to F-actin have been studied by X-ray crystallography.[11]

Precautions and Disclaimer

This product is 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

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. One publication reports preparation of a ~0.5 mM stock solution of TRITC-phalloidin in DMSO.[12]

Several publications cite storage of stock solutions of TRITC-phalloidin in methanol in the dark at –20 °C,[13,14] although we have ourselves not tested the solution stability of TRITC-phalloidin.

Procedure

The following procedure may serve as a general guideline for staining cells.[15] Final staining solutions in aqueous physiological buffers have a phalloidin concentration range of 0.1-100 µM, with corresponding incubation times of 15 minutes to 72 hours.

1. 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.
4. 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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