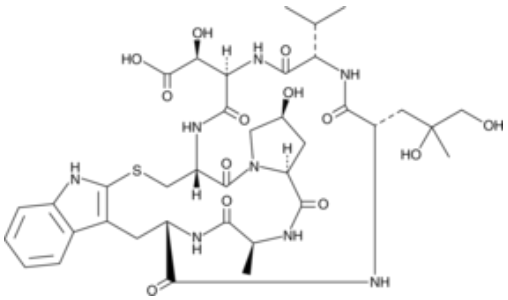
Product Data Sheet

Product Name: Phallacidin
Cat. No.: GC44617

Chemical Properties

Cas. No.	26645-35-2		
SMILES	O=C1[C@@](H])(NC([C@]([C@H](O)C(O)=O)([H])NC([C@H](C(C)C)NC([C@@H]2CC(C)(O)CO)=O)=O)=O)CSC(NC3=C4C=CC=C3)=C4C[C@@H](C(N2)=O)NC([C@H](C)NC([C@@]5([H])N1C[C@@H](O)C5)=O)=O		
Formula	C37H50N8O13S	M.Wt	846.9
Solubility	DMF: Soluble,DMSO: Soluble,Methanol: Soluble,Water: Soluble	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		

Structure



Protocol

Experimental Protocol for Fluorescent Labeling of F-actin Cytoskeleton Using Phallacidin [1]

This protocol is adapted from research data and provided for reference only. Adjustments may be necessary based on specific experimental requirements.

1. Reagent Preparation:

(1) Synthesis of NBD-Phallacidin from Phallacidin: Phallacidin is converted to an amide by reaction

with ethylenediamine. This derivative, containing a primary amine, is then mixed with 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD-Cl) in absolute methanol. The ensuing reaction results in the formation of the fluorescent derivative NBD-Phallacidin. Partial purification is achieved by ether precipitation of the labeled and unlabeled Phallacidin.

(2) NBD-Phallacidin Stock Solution: The NBD-Phallacidin purified by SP-Sephadex chromatography is dissolved in an appropriate buffer (e.g., phosphate-buffered saline or Hanks' balanced salt solution) or cell culture medium to prepare the working solution. For staining fixed cells, the partially purified preparation containing approximately 20% NBD-Phallacidin is used at a working concentration of about 0.9-1.7μg/mL. For live-cell staining, purified NBD-Phallacidin is used at working concentrations of approximately 14-57ng/mL or 5.7-8.6μg/mL.

(3) Buffers and Culture Media: Phosphate-buffered saline (PBS); Hanks' balanced salt solution (HBSS); High glucose buffer A (120mM NaCl, 15mM glucose, 10mM Hepes, 4.4mM $NaHCO_3$, 3mM KCl, 1mM Na_2PO_4, pH=7.1); Dulbecco's modified Eagle's medium (with 10% fetal calf serum).

2. Cell Fixation and Staining:

(1) Cell Fixation: Cells grown on glass coverslips are fixed with 2% formaldehyde (in PBS) for 20 minutes at 22°C.

(2) Permeabilization Treatment: After washing twice with PBS, cells are extracted with pre-cooled acetone (-20°C) for 7-10 minutes and then air-dried.

(3) Staining: Add 350μL of PBS or HBSS containing 0.3-0.6μg of NBD-Phallacidin to the coverslip, and stain for 5-30 minutes.

(4) Washing and Observation: Wash four times with buffer at 4°C to remove unbound toxin, followed by fluorescence microscopic observation.

3. Live Animal Cell Staining Procedure:

(1) Cell Permeabilization: Use the lysolecithin permeabilization method. Treat cells with high glucose buffer A containing 14-40μg/mL palmitoyl lysophosphatidyl choline for 2 minutes.

(2) Buffer System: Wash cells four times with high glucose buffer A to remove lysolecithin. Add 350μL of buffer A containing 5ng of NBD-Phallacidin and stain at room temperature for 5 minutes.

After staining, wash three times with buffer A and observe within 1 hour.

(3) Culture Medium System: Wash cells once with high glucose buffer A. Add Dulbecco's modified medium (with 10% fetal calf serum) containing 2-3μg of NBD-Phallacidin, and stain in a 37°C incubator for 5 minutes. Subsequently, transfer the cells to pre-warmed complete medium for continued culture.

(4) Viability Test: Fluorescein diacetate staining can be used to assess cell membrane integrity and viability.

Precautions:

(1) This method can be used to label various known F-actin structures, including stress fibers, ruffles, the cellular geodome, diffuse membrane-associated actin, and actin cables in algae. Cell nuclei are not stained.

(2) For your safety and health, please wear a lab coat and disposable gloves during operation.

References:
[1] Barak LS, Yocum RR, Nothnagel EA, et al. Fluorescence staining of the actin cytoskeleton in living cells with 7-nitrobenz-2-oxa-1,3-diazole-phallacidin. Proc Natl Acad Sci U S A. 1980 Feb;77(2):980-4.

Background

Phallacidin is a bicyclic heptapeptide toxin derived from poisonous mushrooms. Phallacidin stabilizes microfilament structures and inhibits actin depolymerization by binding with high affinity to filamentous actin (F-actin), thereby disrupting cytoskeletal dynamics. Phallacidin can be used for cytoskeleton visualization research, fluorescent probe development, and studies on microfilament dynamics mechanisms[1-4].

References:
[1] Schmid E, Gu S, Yang W, et al. Serum- and glucocorticoid-inducible kinase SGK1 regulates reorganization of actin cytoskeleton in mast cells upon degranulation. Am J Physiol Cell Physiol. 2013 Jan 1;304(1):C49-55.
[2] Franzén LE. The perforated mesentery of the rat: a novel model for the study of genuine connective tissue contraction. J Surg Res. 1996 Jan;60(1):91-100.

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[3] Carley WW, Barak LS, Webb WW. F-actin aggregates in transformed cells. J Cell Biol. 1981 Sep;90(3):797-802.
[4] Hinshaw DB, Sklar LA, Bohl B, et al. Cytoskeletal and morphologic impact of cellular oxidant injury. Am J Pathol. 1986 Jun;123(3):454-64.

Caution: Product has not been fully validated for medical applications. For research use only.
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