

Product Data Sheet

Product Name: Filipin III
 Cat. No.: GC12048

Chemical Properties

Cas. No. 480-49-9

Chemical Name (3R,4S,6S,8S,10R,12R,14R,16S,17E,19E,21E,23E,25E,27S,28R)-4,6,8,10,12,14,16,27-octahydroxy-3-((R)-1-hydroxyhexyl)-17,28-dimethyloxacyclooctacos-17,19,21,23,25-pentaen-2-one

SMILES O[C@@H]1/C(C)=C/C=C/C=C/C=C/C=C/[C@H](O)[C@@H](C)OC([C@@H])([C@H](O)CCCC)([H])[C@@H](O)C[C@@H](O)C[C@@H](O)C[C@@H](O)C[C@@H](O)C[C@@H](O)C1=O

Formula C₃₅H₅₈O₁₁ M.Wt 654.83

Solubility 1mg/ml in Ethanol; 5mg/ml in DMF; 10mg/ml in DMSO
 Storage Store at -20°C, protect from light. We recommend using the stock solution within 24 hours or it may result in reduced activity.

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.

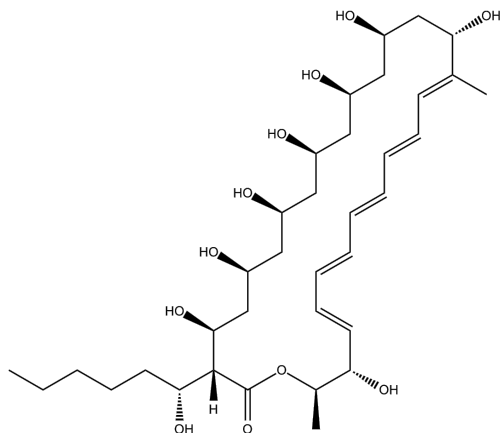
Caution: Product has not been fully validated for medical applications. For research use only.

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Structure



Protocol

This plan only provides a guide, please modify it to meet your specific needs.

1. Preparation of staining solution

(1) Prepare stock solution: Balance the cryogenic solid at room temperature for at least 20min. Centrifuge the tube and concentrate the powder at the bottom of the tube. Dissolve Filipin III in anhydrous DMSO or anhydrous ethanol in a clean bench to prepare a stock solution with a concentration of 1-10mM.

Attention: Filipin III solution is very sensitive to light and air. It is recommended to divide the prepared stock solution into small sizes and fill them with inert gas. Store the stock solution in the dark at -20°C or -80°C to avoid repeated freezing and thawing.

(2) Prepare working solution: Dilute the stock solution with a suitable buffer (such as PBS, HBSS, or other physiological buffer) to prepare a working solution with a concentration of 1-250µM.

Note: Please adjust the concentration of the working fluid according to the actual situation and prepare it now.

2. Cell staining

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- (1) The cells were washed with PBS 2-3 times after absorption of the culture-medium.
- (2) The cells were fixed with 4% Paraformaldehyde at room temperature for 15-20min.
- (3) After fixing, use PBS to clean 2-3 times, 5min each time.
- (4) Add Filipin III working solution into the culture hole, completely cover the cells, and incubate at room temperature for 30min-2h away from light. The optimal incubation time of different cells is different, please adjust it according to the specific experimental needs and dyeing effect.
- (5) After the end of incubation, absorb and discard the dye working solution, and wash the cells with PBS for 2-3 times.

3. Tissue sections staining (frozen sections) :

- (1) Place the sections in a ventilated place and dry at room temperature for 20-30min.
- (2) Immerse the slices in 4% Paraformaldehyde and fix at room temperature for 30min.
- (3) Carefully absorb and discard the fixing solution, and clean the sections with PBS for 2-3 times, 5min each time.
- (4) Drizzle Filipin III working solution onto the sections, cover the sections completely, and incubate at room temperature for 30min-2h away from light. Adjust the dyeing scheme according to the actual situation.
- (5) After the end of incubation, absorb and discard the dye working solution, and wash the cells with PBS for 2-3 times.
- (6) If it is necessary to seal the sections, it is recommended to use water-based fluorescent sealing agent.

4. Microscope detection: The excitation light range of Filipin III is 340-380nm, and the emission light range is 385-470nm.

Precautions:

1. Filipin III has a very high affinity for binding to cholesterol, but it has the characteristic of rapid photobleaching. It is recommended to observe immediately after staining

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2. Filipin III only produces moderate natural fluorescence under UV excitation, and its fluorescence intensity is weaker compared to other fluorescent probes. This should be noted when using confocal microscopy for observation.
3. Fluorescent dyes all have quenching issues, please try to avoid light as much as possible to slow down fluorescence quenching.
4. For your safety and health, please wear lab coats and disposable gloves when operating.

Background

Cholesterol is both an important structural component of cell membranes and an early intermediate in hormone and bile acid biosynthesis. The localization and measurement of cholesterol in cells is therefore of great interest. Filipin is the collective name given to four isomeric polyene macrolides isolated from cultures of *S. filipinensis*; Filipin III is the predominant isomer and the one used in most studies. Filipin binds to cholesterol in membranes, forming ultrastructural aggregates and complexes which can be visualized by freeze-fracture electron microscopy.[1],[2] The binding of cholesterol also decreases the intrinsic fluorescence of Filipin, and this property has also been used to detect cholesterol in membrane fractions.[3]

Reference:

- [1]. Castanho, M.A., Coutinho, A., and Prieto, M.J. Absorption and fluorescence spectra of polyene antibiotics in the presence of cholesterol J. Biol. Chem. 267(1), 204-209 (1992).
- [2]. Miller, R.G. Mini Review. The use and abuse of filipin to localize cholesterol in membranes Cell Biol. Int. Rep. 8(7), 519-535 (1984).
- [3]. Severs, N.J., and Robenek, H. Detection of microdomains in biomembranes. An appraisal of recent developments in freeze-fracture cytochemistry Biochimica et Biophysica Acta 737, 373-408 (1983)

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