

## Product Data Sheet

Product Name: Cholesterol-13C2

Cat. No.: GC64477

### Chemical Properties

Cas. No. 78887-48-6

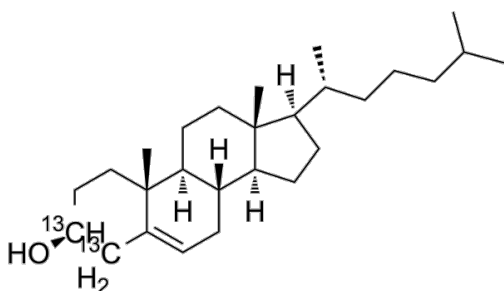
Formula  $C_{25}^{13}C_2H_{46}O$  M.Wt 388.64

Solubility Ethanol : 5.56 mg/mL (14.31 mM; Need ultrasonic) 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

Cholesterol-13C2 is used in cholesterol uptake experiments [1].

This experimental protocol is compiled based on the research content. The protocol is for reference only. Please adjust the protocol according to actual needs.

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

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(1) Reagent preparation: Dissolve Cholesterol-13C2 in chloroform to prepare a stock solution. Use 20µL olive oil to prepare a solution containing 540µmol Cholesterol-13C2. Dissolve Cholesterol-13C2 in chloroform to prepare an internal standard solution at 0.4µg/20µL. Prepare 150mol/L sodium chloride solution. Prepare chloroform/methanol (2:1; v/v) extraction solution. Prepare hexane/isopropanol (95:5; v/v) as liquid chromatography mobile phase.

(2) Cell culture and spiking: Seed and culture Caco-2 cells to 12-14 days post-confluency monolayers.

(3) In vitro digestion and sample preparation: Take 2.0mL Ensure, 2.0mL orange juice (with or without phytosterols), and 20µL olive oil containing 540µmol Cholesterol-13C2. Perform simulated gastric and small intestinal digestion. After digestion, homogenize and centrifuge at 150000g, 4°C for 35min. Filter the supernatant through 0.22µm to collect. Dilute with Dulbecco's minimal essential medium at 1:4. Add to Caco-2 cell monolayers and culture for 4h (37°C, 5% CO2).

(4) Cell washing and collection: Remove medium. Wash cell monolayers twice with ice-cold phosphate-buffered saline (PBS) containing 2g/L albumin. Wash twice with ice-cold PBS without albumin. Collect cells in 1.5mL ice-cold PBS. Centrifuge at 1800rpm, 4°C for 5min. Store cell pellets at -80°C under nitrogen.

(5) Cholesterol extraction: Thaw cell pellets. Add 0.3mL 150mol/L sodium chloride. Sonicate on ice (setting 3, 10s). Add 1.2mL chloroform/methanol (2:1) to extract cholesterol. Vortex. Centrifuge at 2000g to accelerate phase separation. Repeat extraction twice. Combine chloroform layers (lower layers). Dry under nitrogen stream. Dissolve residue in 1mL hexane. Filter through 0.2µm membrane. Use for LC/APCI-MS/MS analysis.

### Precautions:

(1) The method detection limit is 2.2pmol. The lower limit of quantification is 7.2pmol. The linear range is 7.2-1127pmol. The regression coefficient  $R > 0.997$ .

(2) Cholesterol-13C2 is stable in hexane at room temperature for 2 weeks.

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

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[1] Tian Q, Failla ML, Bohn T, et al. High-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry determination of cholesterol uptake by Caco-2 cells. *Rapid Commun Mass Spectrom.* 2006;20(20):3056-60.

### Background

Cholesterol-13C2 is a 13C-labeled cholesterol. Cholesterol-13C2 serves as an internal standard in mass spectrometry and NMR analyses for accurate quantification and metabolic studies of cholesterol and related metabolites<sup>[1-4]</sup>.

### References:

- [1] Ostlund RE Jr, Matthews DE. [13C]cholesterol as a tracer for studies of cholesterol metabolism in humans. *J Lipid Res.* 1993 Oct;34(10):1825-31.
- [2] Lehmann WD, Kessler M. Accurate quantification of nonesterified and total cholesterol in plasma and urine from crude lipid extracts by stable isotope dilution and field desorption mass spectrometry. *J Clin Chem Clin Biochem.* 1982 Dec;20(12):893-8.
- [3] Shackleton CH, Reid S. Diagnosis of recessive X-linked ichthyosis: quantitative HPLC/mass spectrometric analysis of plasma for cholesterol sulfate. *Clin Chem.* 1989 Sep;35(9):1906-10.
- [4] Ferezou J, Sulpice JC, Coste T, et al. Origins of neutral sterols in human feces studied by stable isotope labeling (D and 13C). Effect of phytosterols and calcium. *Digestion.* 1982;25(3):164-72.

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