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Product Information

Cholesterol Extraction Kit

Catalog Number **MAK175**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description

Cholesterol is a structural component of cell membranes and a precursor to hormones. The Cholesterol Extraction Kit enables the extraction of sterols such as cholesterol from biological samples in two simple steps. The extracted cholesterol can then be saponified, derivatized, and quantified using gas chromatography (GC) with flame-ionization detection (FID) or gas chromatography/mass spectrometry (GC/MS).

Similar to fatty acids, sterols including cholesterol are typically extracted from biological samples using chloroform, methanol, and water to separate lipids from polar compounds.[1] Lipids are retained in the lower chloroform phase; whereas, polar compounds are retained in the methanol-water layer. The sample is then centrifuged and the bottom chloroform layer containing cholesterol is transferred with a pipette to another test tube. An aliquot of the transferred layer is saponified with methanolic NaOH or KOH, and then derivatized with trimethylsilyl chloride (chlorotrimethyl-silane).[2,3] The formed cholesterol-TMS ester is reconstituted with hexane and injected directly into a GC-FID or GC/MS system for quantification.

The Cholesterol Extraction Kit shortens the extraction process by eliminating the need to prepare solvents and standards, centrifugation, and pipetting. Once the sample is homogenized and dissolved in the Extraction Solvent containing the internal standard, it is vortexed and poured into the syringe containing a filter, which preferentially elutes the chloroform layer containing cholesterol. The user then squeezes the plunger to ensure that cholesterol is eluted from the syringe filter. A portion of the total cholesterol extract can then be saponified and derivatized for GC-FID or GC/MS analysis as described in the Procedure. Data comparing the standard Folch method to the Cholesterol Extraction Kit extraction method are presented in the Results Section.

Components

The kit is sufficient for 40 extractions.

Extraction Solvent containing 0.05 mg/mL of 5-α-cholestane as an internal standard Catalog Number MAK175A	123 mL
Aqueous Buffer Catalog Number MAK175B	40 mL
Plunger Syringe w/ Filter Catalog Number MAK175C	40 each

Reagents and Equipment Required but Not Provided

- Homogenizer to homogenize solid samples
- Capped Pyrex® glass tubes to collect the total sterol extract
- Gas chromatography system (GC), preferably with a flame-ionization detector (FID)
- Polar gas chromatography column
- Sodium hydroxide (Catalog Number S8045 or equivalent) **OR** Potassium hydroxide (Catalog Number P5958 or equivalent)
- Methanol (Catalog Number 1.06011 or equivalent)
- Hexane (Catalog Number 227064 or equivalent)
- Chlorotrimethylsilane (Catalog Number 89595 or equivalent)

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

This kit is shipped at ambient temperature. Storage at room temperature is recommended.

Procedure

Sample Preparation

1. Add 3 mL of Extraction Solvent to each sample. Cholesterol can be extracted from up to 0.15 g of sample containing <10% lipids.
   Note: If using whole blood (10-50 µL), it is not necessary to correct for water content with Aqueous Buffer in Step 3.

2. Homogenize in Extraction Solvent if the sample is a solid and vortex. If the sample is liquid (e.g. plasma), simply vortex.

3. Add 0.5 mL of Aqueous Buffer and vortex.
   Note: If the sample is a liquid (e.g. blood serum or plasma), the buffer amount will have to be amended so the total volume of the aqueous solution is 0.5 mL. For example, if 0.2 mL of serum sample is mixed with 3 mL of Extraction Solvent in step 1, add 0.3 mL of buffer to bring the aqueous volume to 0.5 mL.

4. Place the syringe containing the filter on top of a collecting tube that can hold at least 2 mL of liquid.

5. Pour the solution into the syringe, attach plunger, and push the plunger to elute cholesterol into the collecting tube. The eluted solvent contains the cholesterol extract.
   Note: Avoid excessive plunging. Although the filter selectively traps water in the apparatus, excessive plunging may inadvertently force water though the filter.

6. The extracted cholesterol may now be saponified, derivatized, and analyzed by GC-FID or GC/MS.

Recommended Saponification Procedure

1. Aliquot ~200 µL of the total cholesterol extract from Sample Preparation, step 5 and dry under nitrogen.

2. After drying, add 3 mL of 1 M methanolic NaOH or methanolic KOH, and heat at 90 °C for 1 hour.

3. Cool for 10 minutes.

4. Add 2 mL of 0.9% saline and 5 mL of hexane, vortex, and centrifuge at 500 × *g*.

5. Transfer top hexane layer to a new test tube. Add 5 mL of hexane, vortex, and centrifuge at 500 × *g*.

6. Pool the upper hexane layer with the first hexane extract (see step 5) and proceed to derivatization.

Recommended Derivatization Procedure

1. Aliquot ~1 mL of hexane containing cholesterol and dry under nitrogen.

2. Add 0.3 mL of trimethylsilyl (TMS) chloride to the dried cholesterol extract. Heat at 60 °C for 30 minutes.

3. Dry under nitrogen.

4. Reconstitute the derivatized cholesterol in 50-100 µL of hexane and transfer to a GC vial. Inject into a GC-FID or GC/MS system with an appropriate non-polar column.[3]

Results

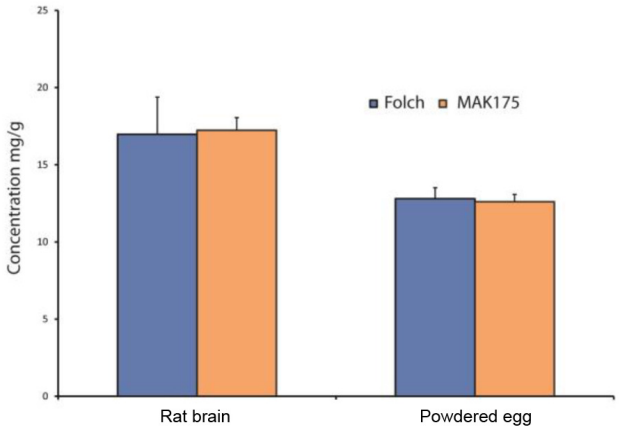
1. Calculation of GC-FID Results
   Concentration (mg/g) of cholesterol in sample equals:

$$\frac{\text{Amount of internal standard (mg)} \times \text{Area of sample Cholesterol peak}}{\text{Area of internal standard} \times \text{Weight of tissue (g)}}$$

   Amount of internal standard = 0.15 mg per sample (when using 3 mL of Extraction Solvent containing 5-α-cholestane per sample).

2. Data comparing Folch standard method to MAK175 Kit method

Figure 1.
Rat brain and powdered egg cholesterol concentrations (mg/g).



Lipids were extracted with the Folch or MAK175 Kit method, saponified, derivatized, and quantified with GC-FID.

References

1. Folch, J. et al., A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., **226**, 497-509 (1957).
2. Adams, M.L. et al., Evaluation of direct saponification method for determination of cholesterol in meats. Journal - Association of Official Analytical Chemists, **69**, 844-6 (1986).
3. Taha, A.Y. et al., Brainstem concentrations of cholesterol are not influenced by genetic ablation of the low-density lipoprotein receptor. Neurochemical Research, **34**, 311-5 (2009).

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