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 including cholesterol from biological samples in two simple steps. The extracted sterols 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 using a dual solvent partition system containing a lipophilic solvent and an aqueous solvent according to a published procedure. Chloroform, methanol, and water are used to separate lipids/sterols from aqueous-soluble compounds. Lipids/sterols are retained in the lower chloroform phase; whereas, aqueous-soluble compounds are retained in the methanol-water layer. The sample is then centrifuged and the bottom chloroform layer is transferred with a pipette to another test tube. An aliquot of the transferred layer is then saponified with methanolic NaOH or KOH, and then derivatized with trimethylsilyl chloride (chlorotrimethylsilane). 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 inverted twice and poured into the syringe containing a filter, which preferentially elutes the chloroform layer containing sterols. The user has to squeeze the plunger to ensure most of the sterols are eluted. After that, a portion of the total sterol extract can 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 is presented.

Components
The kit is sufficient for 40 extractions.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction Solvent</td>
<td>120 mL</td>
</tr>
<tr>
<td>Catalog Number MAK175A</td>
<td></td>
</tr>
<tr>
<td>Aqueous Buffer</td>
<td>40 mL</td>
</tr>
<tr>
<td>Catalog Number MAK175B</td>
<td></td>
</tr>
<tr>
<td>Filter</td>
<td>40 each</td>
</tr>
<tr>
<td>Catalog Number MAK175C</td>
<td></td>
</tr>
<tr>
<td>Plunger</td>
<td>40 each</td>
</tr>
<tr>
<td>Catalog Number MAK175D</td>
<td></td>
</tr>
</tbody>
</table>

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. Sterols can be extracted from up to 0.15 g of sample containing <10% lipids.

2. Homogenize if the sample is a solid and vortex. **Note:** The Extraction Solvent can also be added after homogenizing the sample.

3. Add 0.5 mL of Aqueous Buffer. Invert twice or vortex.

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 sterols into the collecting tube. The eluted solvent contains the total sterol extract. **Note:** Avoid excessive plunging. Although the filter selectively traps water in the apparatus, excessive plunging may inadvertently force water through the filter.

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

**Saponification**

1. Aliquot ~200 µL of the total sterol 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.

**Derivatization**

1. Aliquot ~1 mL of the saponified material and dry under nitrogen.

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

3. Dry under nitrogen.

4. Reconstitute the derivatized sterols in 50–100 µL of hexane and transfer to a GC vial. Inject into a GC-FID or GC/MS system with appropriate column.
Results

1. **Calculation of GC-FID Results**
   Concentration (mg/g) of sterols in sample equals:
   
   \[
   \frac{\text{Amount of internal standard (mg)} \times \text{Area of sample Sterol peak}}{\text{Area of internal standard} \times \text{Weight of tissue (g)}}
   \]
   
   Amount of internal standard = 0.15 mg per sample when using the Extraction Solvent, which contains an internal standard.

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


Pyrex is a registered trademark of Corning, Inc.

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