Cholesterol extraction kit

Catalog number: 20095
40 Tests
For Research Use Only. This kit has not been validated for diagnostic purposes.

Certo Labs Inc.
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1. Intended purpose

The Certo Labs cholesterol extraction kit is intended for the extraction of cholesterol and cholesterol-like compounds (sterols) from biological tissue and food samples.

2. Introduction

Cholesterol is a structural component of cell membranes and a precursor to hormones. The Certo Lipids extraction kit enables the extraction of sterols containing cholesterol from biological samples in two simple steps. The extracted sterols can then be saponified, derivitized and quantified using gas-chromatography (GC) with flame-ionization detection (FID) or gas-chromatography mass spectrometry (GCMS).

3. Principle:

General principle: Homogenize → Extract → Saponify → Derivitize → Run on GC-FID or GCMS.

Similar to fatty acids, sterols containing cholesterol are extracted using a dual solvent partition system containing a lipophilic solvent and an aqueous solvent (Folch et al., 1957). Chloroform, methanol and water are used to separate lipids containing sterols from aqueous-soluble compounds. Lipids are retained in the lower chloroform phase, whereas aqueous-soluble compounds are retained in the methanol-water layer. The sample is then centrifuged, to achieve uniform separation, and the bottom chloroform layer is transferred with a pipette to another test-tube. An aliquot of that transfer is then hydrolyzed with 1M methanolic NaOH or KOH at 90ºC for 1 hour, as described by Adams et al. (Adams et al., 1986). The resulting free cholesterol is extracted with 2 ml saline and twice with 5 ml hexane. The hexane layer is separated, dried and derivitized with trimethylsilyl (TMS) chloride at 60ºC for 30 min (Taha et al., 2009). The TMS reagent is then dried down under nitrogen and the formed cholesterol-TMS ester is reconstituted with 50-100 µl hexane and injected directly into a GC-FID or GCMS system for quantitation.

The Certo Sterol Extraction kit shortcuts the extraction process by eliminating the need to prepare solvents and standards, centrifuge and pipette. Once the sample is homogenized and dissolved in Certo's solvent containing the internal standard, it is inverted twice and poured into the syringe containing the filter, which preferentially elutes the chloroform layer containing sterols. The user has to squeeze the plunger to make sure that most of the lipids are eluted. After that, a portion of the total lipid extract can be sent back to Certo Labs for analysis, or saponified and derivitized for GC-FID or

GCMS analysis as described below in sections 10, 11 and 12. Data comparing the Folch standard method to the Certo kit extraction method is presented in section 15.

4. Warnings and precautions

- All reagents within the Certo lipid extraction kit are intended for research purposes.
- The kit contains chloroform and methanol, which are highly flammable and toxic if inhaled or contacted with the skin. These solvents must be handled with gloves, eye protection, appropriate protective clothing and under a fumehood. In case of an accident, contact a physician immediately.
- Wear gloves while handling the kit and wash hands thoroughly afterwards.
- Do NOT pipette by mouth.
- Do not eat, drink, smoke or apply makeup in areas where the kit is handled.

5. Materials provided

Article	Designation	Amount
Extraction solvent containing 5α–cholestane internal standard	**Chloroform / methanol (2:1 v/v) containing 0.15mg of 5α-cholestane per sample (18mg total) as an internal standard**	**1 x 120 ml**
Buffer	**Aqueous buffer**	**40 ml**
Syringe containing filter	**Filter**	**40**
Syringe plunger	**Plunger**	**40**

6. Additional special equipment

- Homogenizer to homogenize solid samples.
- Pyrex glass tubes to collect the total lipid extract.
- A GC system with a flame-ionization detector (FID) or GCMS system.
- Polar gas-chromatography column

7. Reagent preparation

None.

8. Specimen

Any biological sample – food or animal tissue.

9. Procedure

The Certo kit procedure enables the rapid extraction of sterols from biological samples for quantitation by GC or GCMS.

10. Sample preparation

1. Add 3 ml of Solvent (containing internal standard) to each sample. Sterols can be extracted from up to 0.15g of sample containing <10% lipids.
2. Homogenize if the sample is a solid sample. Note that the solvent can also be added after homogenizing the sample.
3. Vortex.
4. Add 0.5 ml of buffer.
5. Invert twice or vortex.
6. Pour the solution into the syringe containing the filter. *NOTE: The syringe should be placed on top of a collecting tube (Falcon tube, pyrex tube, etc.) that can hold at least 2 ml of solvent.*
7. Push the plunger to elute sterols into the collecting tube. The eluted solvent contains total sterols. *CAUTION: Although the filter selectively traps water in the apparatus, however, excessive plunging may inadvertently force water through the filter.*
8. The extracted sterols may now be:
    a. Sent to Certo Labs for analysis by GC-FID or GCMS. *NOTE: Contact Certo Labs Inc. for more information (1-855-607-0747).*
    b. Saponified, derivitized, and stored in GC vials that can be sent to Certo Labs Inc. for GC-FID or GCMS analysis. *NOTE: Contact Certo Labs Inc. for more information.*
    c. Saponified and derivitized as described below (sections 11 and 12), and analyzed by GC-FID or GCMS.

11. Saponification

1. A portion of the total lipid extract (200 µl) can be aliquoted and dried under nitrogen.
2. After drying, add 3 ml of 1M methanolic sodium hydroxide (or methanolic potassium hydroxide).
3. Heat for 1 hour at 90ºC.
4. Allow to cool for 10 minutes.
5. Add 2 ml of 0.9% saline and 5 ml hexane.
6. Vortex and centrifuge at 500 g.
7. Transfer top hexane layer to a new test-tube.
8. Add 5 ml hexane.
9. Vortex and centrifuge at 500 g.
10. Pool the top hexane layer with the first hexane extract (from step 7) and proceed to derivitization (section 12).

12. Derivitization

1. A portion of the saponified material (1mL) can be aliquoted and dried under nitrogen.
2. Add 0.3 ml trimethylsilyl chloride (TMS) to the dried sterol extract.
3. Heat at 60°C for 30 minutes.
4. Dry the TMS under nitrogen.
5. Reconstitute the derivitized sterols in 50-100 μL hexane and transfer to a GC vial.
6. Inject into a GC-FID or GCMS system with appropriate column (Taha et al., 2009).

13. Calculation of GC-FID results

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

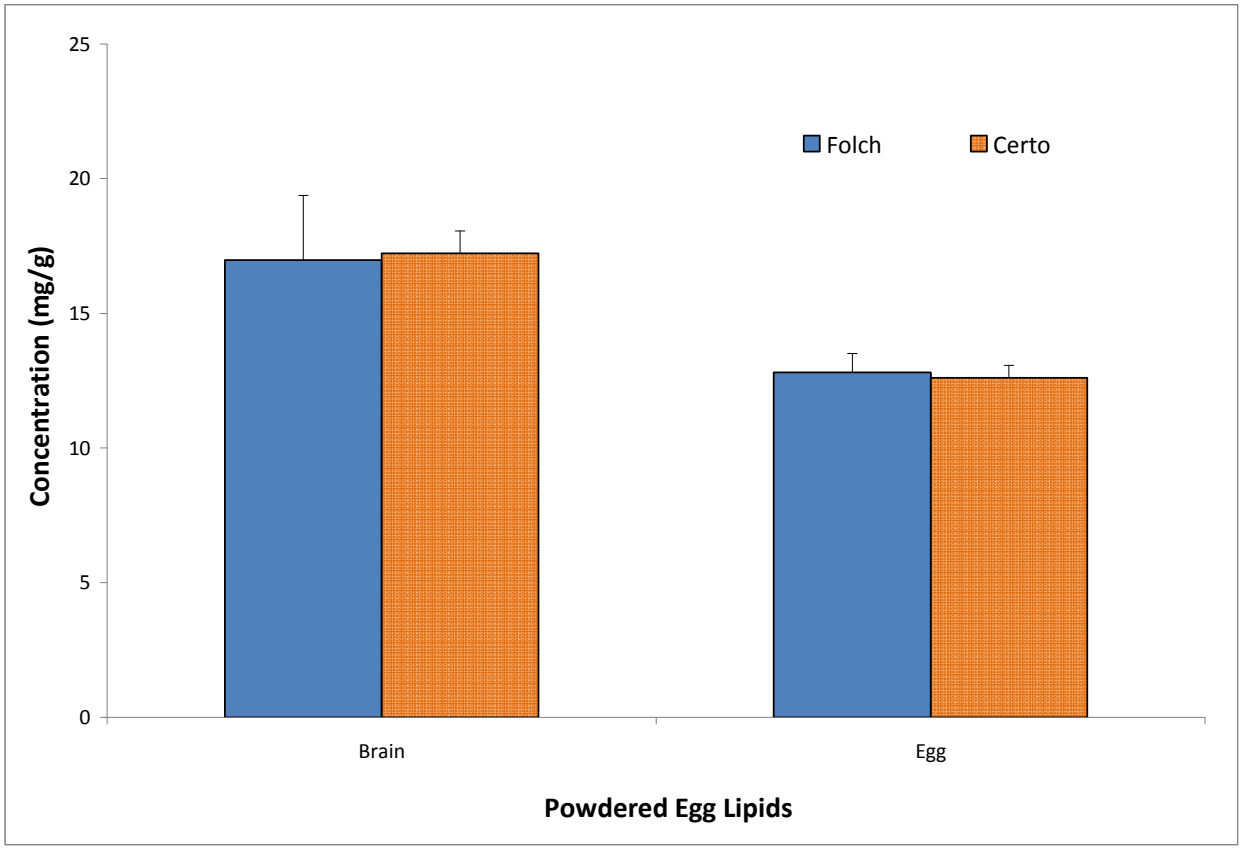
Amount of internal standard = 0.15 mg per sample using the Certo kit solvent, which contains an internal standard.

14. Disposal

The syringe, filter and plunger must be disposed in appropriate chemical waste containers after use. They cannot be used more than once.

15. Data comparing Folch standard method to the Certo kit method.

Figure 1: Rat brain and powdered egg cholesterol concentrations (mg / g). Lipids were extracted with the Folch or Certo kit method, saponified, derivitized and quantified with GC-FID.



16. References

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