Lipid (fatty acid) extraction kit

Catalog number: 20094
40 Tests
For Research Use Only. This kit has not been validated for diagnostic purposes.

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1. Intended purpose

The Certo Labs lipid extraction kit is intended for the extraction of lipids (fatty acids) from biological tissue, food samples and oil.

2. Introduction

Lipids are structural components of cell membranes that play a critical role in gene transcription, signaling and metabolism. Several lipid species exist in biological systems, including phospholipids, cholesterol, triglycerides and fatty acids. The Certo Lipids extraction kit enables the extraction of these lipids in one step. The extracted lipids can then be transesterified and quantified using gas-chromatography (GC) with flame-ionization detection (FID).

3. Principle:

General principle: Homogenize → Extract → Transesterify → Run on GC-FID

Lipids are typically extracted using a dual solvent partition system containing a lipophilic solvent and an aqueous solvent. The Folch method (Folch et al., 1957) has been conventionally used to extract lipids from biological samples, using chloroform, methanol and water to separate lipids from aqueous-soluble compounds. In this procedure, lipids are retained in the lower chloroform layer, whereas aqueous-soluble compounds are retained in the upper 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 transesterified with 14% boron trifluoride, 1% sulphuric acid in methanol, 1% hydrochloric acid in methanol or sodium methoxide. This transesterification reaction results in fatty acid methyl esters (R-COOH→R-COCH3) that can be separated from the transesterification medium with water and heptane or hexane, and injected directly into a GC-FID system for quantitation.

The Certo Lipid 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 a filter, which preferentially elutes the chloroform layer containing total lipids. The user then has to squeeze the plunger to make sure that the lipids are eluted. After that, a portion of the total lipid extract can be sent back to Certo Labs for analysis, or transesterified for GC-FID analysis as described below in sections 10 and 11. Data comparing the standard Folch method to the Certo kit extraction method is presented in section 14.

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 nonadecanoic acid ethyl ester internal standard*	**Chloroform / methanol (2:1 v/v) containing 0.15 mg per 3 ml (6 mg total) of nonadecanoic acid ethyl ester as an internal standard**	**1 x 120 ml**
Buffer	**Aqueous buffer**	**40 ml**
Syringe containing filter	**Filter**	**40**
Syringe plunger	**Plunger**	**40**

*Solvent also contains 0.15 mg of 5-alpa cholestane per 3 ml chloroform / methanol, in case the user wants to measure cholesterol.

6. Additional special equipment

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

7. Reagent preparation

None.

8. Specimen

Any biological sample – food, oil or animal tissue. It is recommended that the kit be validated against the lab's standard protocol and specific tissue matrix, before being used for routine analysis.

9. Procedure

Fatty acid concentrations or percent composition in total lipid extracts can be derived from biological specimens. The Certo kit enables the extraction of total lipids for fatty acid quantitation by GC-FID.

10. Sample preparation

1. Weight the sample.
2. Add 3 ml of Solvent (contains internal standard) to each sample. Lipids can be extracted from up to 0.15g of sample containing <10% lipids, or 0.01 g for adipose tissue, or < 5 mg for oil.
3. Homogenize if the sample is a solid sample. Note that the solvent can also be added after homogenizing the sample.
4. Vortex.
5. Add 0.5 ml of buffer.
6. Invert twice or vortex.
7. 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.*
8. Push the plunger to elute lipids into the collecting tube. The eluted solvent contains total lipids. *CAUTION: Although the filter selectively traps water in the apparatus, excessive plunging may inadvertently force water through the filter.*
9. A portion of the total lipid extract may now be transesterified as suggested in Section 11.

11. Transesterification procedure

1. A portion of the total lipid extract (100 µl) can be aliquoted and dried under nitrogen for transesterification.
2. After drying, add 1 ml of 1% H_2SO_4 in methanol + 0.5 ml hexane, cap and heat at 70ºC for 3 hours OR add 1 ml 14% boron trifluoride + 0.3 ml hexane, cap and heat at 95°C for 1 hour.
3. Add 1 ml hexane and 1 ml distilled water to 14% BF_3 in methanol OR add 1 ml hexane and 1 ml 5% NaCl to 1% H_2SO_4
4. Vortex and centrifuge at 500 g for 5 minutes.
5. Transfer the top hexane layer, dry under nitrogen, re-aliquot under 65-100 µL hexane and add to a GC vial and inject into a GC-FID system with appropriate column. GCMS can also be used for quantitation, following determination of the response factor for each fatty acid.

12. Calculation of GC-FID results

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

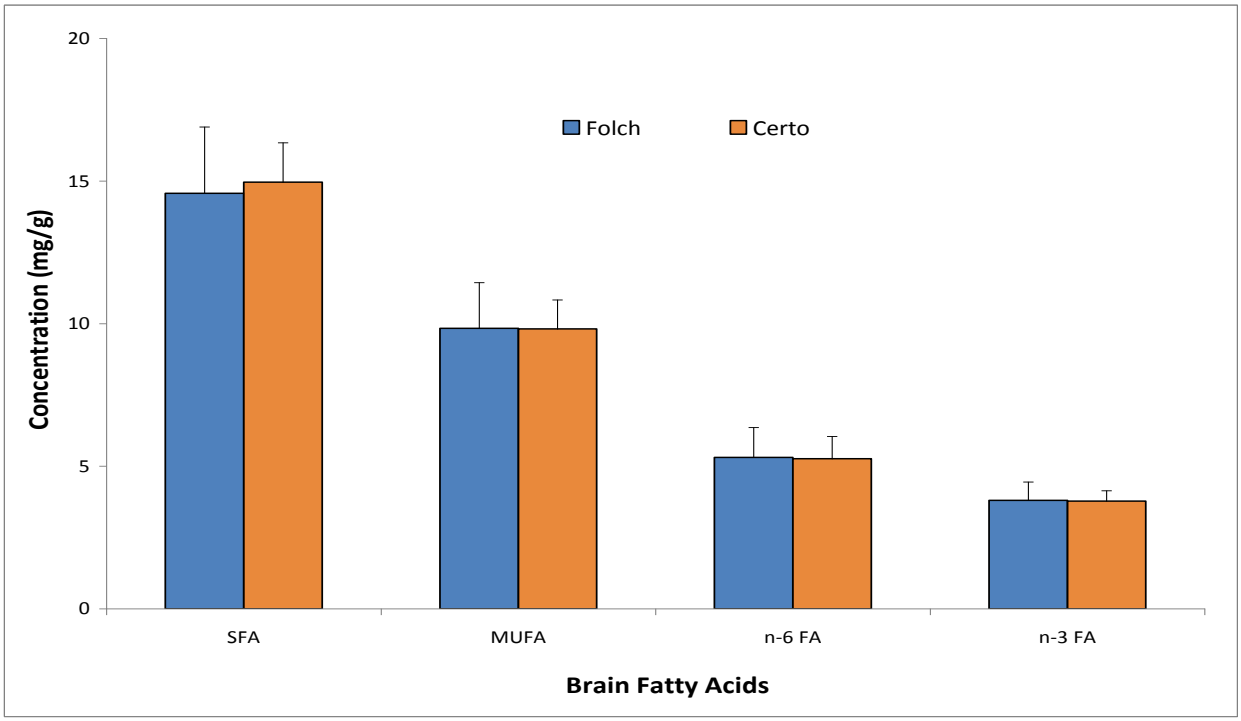
Amount of internal standard = 0.15 mg per sample using the Certo kit solvent, which contains an internal standard.

13. Disposal

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

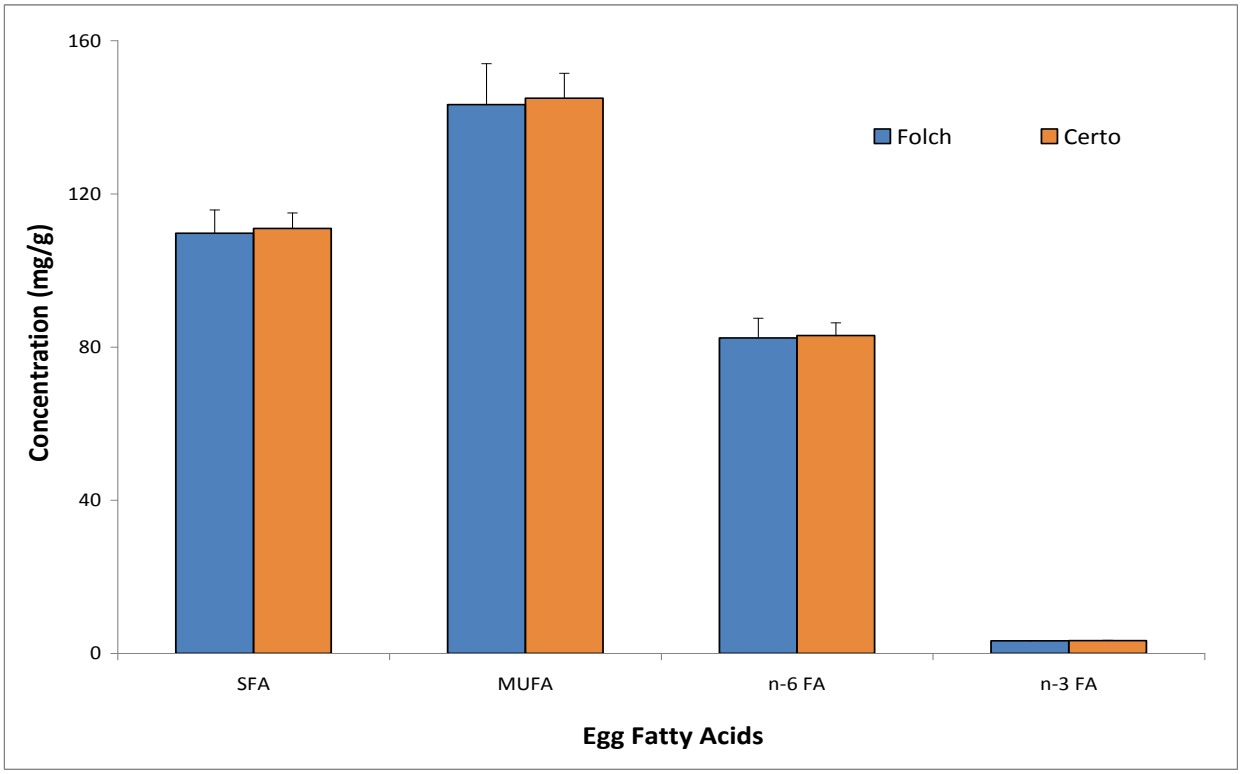
14. Data comparing Folch standard method to the Certo kit method.

Figure 1: Rat brain fatty acid concentrations (mg / g). Lipids were extracted from rat brain with the Folch or Certo kit method, transesterified and quantified with GC-FID.



SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; n-6 FA, omega-6 polyunsaturated fatty acids; n-3 FA, omega-3 polyunsaturated fatty acids.

Figure 2: Powdered egg fatty acid concentrations (mg / g) in rat brain. Lipids were extracted with the Folch or Certo kit method, transesterified and quantified with GC-FID.



SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; n-6 FA, omega-6 polyunsaturated fatty acids; n-3 FA, omega-3 polyunsaturated fatty acids.

15. References

Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipides from animal tissues. The Journal of biological chemistry. 226**,** 497-509.