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Product Information

Fatty Acid Extraction Kit, High Standard

Catalog Number **MAK338**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description

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, triglycerides, and fatty acids. The Fatty Acid 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).

The Folch method has been conventionally used to extract lipids containing fatty acids from biological samples, using chloroform, methanol, and water to separate lipids from aqueous-soluble compounds.[1] 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 the transferred chloroform layer is then transesterified with 14% boron trifluoride in methanol or 1% sulfuric acid in methanol. This transesterification reaction results in fatty acid methyl esters:

$$R\text{-}COOH \rightarrow R\text{-}COOCH_3$$

The methyl esters can be separated from the transesterification medium with water, and heptane or hexane, and injected directly into a GC-FID system for quantitation.

The Fatty Acid Extraction Kit shortens the extraction process by eliminating the need to centrifuge, pipette, and prepare solvents and standards. Once the sample is homogenized and dissolved in the Extraction Solvent containing the internal standard, it is vortexed and poured into the plunger syringe with filter, which preferentially elutes the chloroform layer containing total lipids. The user then squeezes the plunger to ensure that the lipids are eluted from the syringe filter. A portion of the total lipid extract containing the total fatty acids can then be transesterified for GC-FID analysis as described in the Procedure. Data comparing the standard Folch method to the Fatty Acid Extraction Kit extraction method are presented under the Results Section.

When extracting fatty acids at normal or high levels, such as in food matrices, use the MAK338 Fatty Acid Extraction Kit. This kit produces stronger signals. If in doubt about which kit to use, use MAK338. When extracting fatty acids at low or very low levels, such as in cellular matrices, use the MAK174 Fatty Acid Extraction Kit.

Components

The kit is sufficient for 40 extractions.

Extraction Solvent containing 0.15 mg/mL of nonadecanoic acid ethyl ester as an internal standard Catalog Number MAK338A	123 mL
Aqueous Buffer Catalog Number MAK338B	40 mL
Plunger Syringe with Filter Catalog Number MAK338C	40 each

Reagents and Equipment Required but Not Provided.

- Homogenizer to homogenize solid samples
- Capped Pyrex® glass tubes to collect the total lipid extract
- Gas chromatography system (GC), preferably with a flame-ionization detector (FID)
- Polar gas chromatography column
- Sulfuric acid (Catalog Number 258105 or equivalent) in methanol (Catalog Number 1.06011 or equivalent) **OR** Boron trifluoride-methanol solution (Catalog Number B1252 or equivalent)
- Hexane (Catalog Number 227064 or equivalent)

Precautions and Disclaimer
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. Weigh the sample. Add 3 mL of Extraction Solvent to each sample. Lipids can be extracted from up to 0.15 g of sample containing <10% lipids, 0.01 g of adipose tissue, or <5 mg of oil.
   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 add the Extraction Buffer and vortex.
3. Add 0.5 mL of Aqueous Buffer to the homogenized sample 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 homogenized sample into the syringe, attach plunger, and push the plunger to elute lipids into the collecting tube. The eluted solvent contains the total lipid extract.
   Note: Avoid excessive plunging. Although the filter selectively traps water/methanol, excessive plunging may inadvertently force water though the filter.
6. The total lipid extract can be transesterified and analyzed by GC-FID.

Transesterification
1. Aliquot 100 µL of the total lipid extract from Sample Preparation, step 5 and dry under nitrogen for transesterification.
   Note: Preliminary testing may be required to establish the appropriate volume to utilize. Less than 5 mg total lipids per sample is sufficient to ensure efficient transesterification.
2. Two suggested reactions for transesterification:
   a. After drying, add 1 mL of 1% H_2SO4 in methanol and 0.5 mL of hexane. Cap and heat at 70 °C for 3 hours. Add 1 mL of hexane and 1 mL of 5% NaCl.
      OR
   b. Add 1 mL of Boron trifluoride-methanol solution (Catalog Number B1252) and 0.3 mL of hexane. Cap and heat at 95 °C for 1 hour. Add 1 mL of hexane and 1 mL of distilled water.
3. After completing a or b, vortex and centrifuge at 500 × *g* for 5 minutes.
4. Transfer the top hexane layer and dry under nitrogen. Reconstitute the transesterified lipids with 65–100 µL of hexane and add to a GC vial. Inject into a GC-FID system with appropriate column. GC/MS can also be used for quantitation, after establishing response factors for each fatty acid.

Results

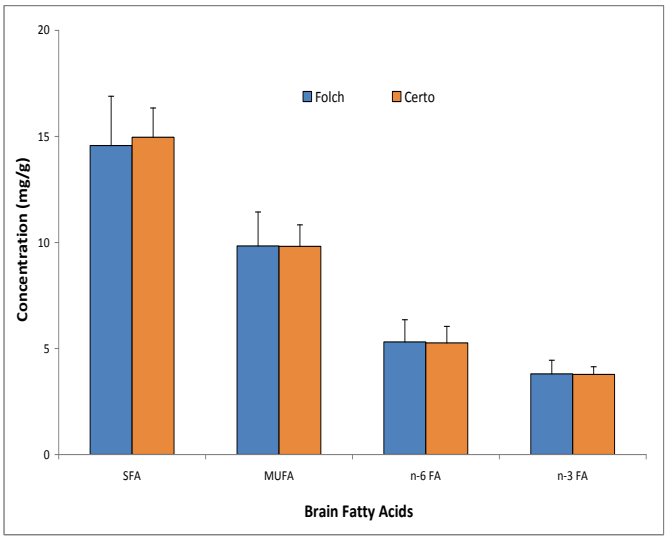
1. Calculation of GC-FID Results
   Concentration (mg of fatty acid/g sample) equals:

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

   Amount of internal standard = 0.45 mg when using 3 mL of Extraction Solvent (per sample), which contains nonadecanoic acid ethyl ester as an internal standard.

2. Data comparing Folch standard method to MAK174/MAK338 Kit method:

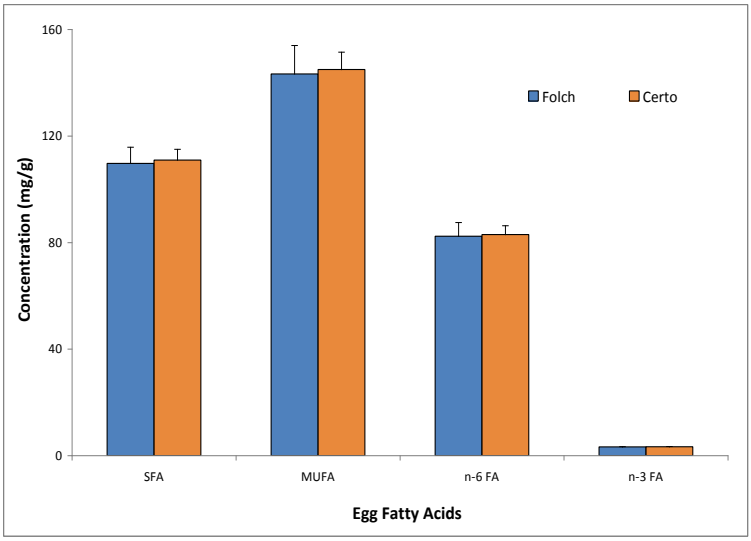
Figure 1.
Rat brain fatty acid concentrations (mg/g)



Lipids were extracted from rat brain with the Folch or MAK174/MAK338 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



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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).

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