

## Product Information

### Fatty Acid Extraction Kit, Low Standard

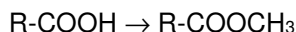
Catalog Number **MAK174**  
Store at Room Temperature

## 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).

Lipids are typically extracted using a dual solvent partition system containing a lipophilic solvent and an aqueous solvent. 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.<sup>1</sup> 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 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:



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 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 total lipids. The user then has to squeeze the plunger to ensure the lipids are eluted.

A portion of the total lipid extract containing 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 is presented.

### Components

The kit is sufficient for 40 extractions.

Extraction Solvent Catalog Number MAK174A	120 mL
Aqueous Buffer Catalog Number MAK174B	40 mL
Plunger Syringe w/ Filter Catalog Number MAK174C	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  $\mu$ L), it is not necessary to correct for water content with Aqueous Buffer in Step 3.

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 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 lipids into the collecting tube. The eluted solvent contains the total lipid extract. Note: Avoid excessive plunging. Although the filter selectively traps water, excessive plunging may inadvertently force water through the filter.
6. The total lipid extract may now be transesterified and analyzed by GC-FID.

Transesterification

1. Aliquot 100  $\mu$ L of the total lipid extract from Sample Preparation, step 5 and dry under nitrogen for transesterification.
2. Two suggested reactions for transesterification:
  - a. After drying, add 1 mL of 1% H<sub>2</sub>SO<sub>4</sub> 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. Vortex and centrifuge at 500  $\times$  g for 5 minutes.
  4. Transfer the top hexane layer and dry under nitrogen. Reconstitute the transesterified lipids with 65–100  $\mu$ L 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, following determination of the response factor for each fatty acid.

## Results

### 1. Calculation of GC-FID Results

Concentration (mg/g) of lipids in sample equals:

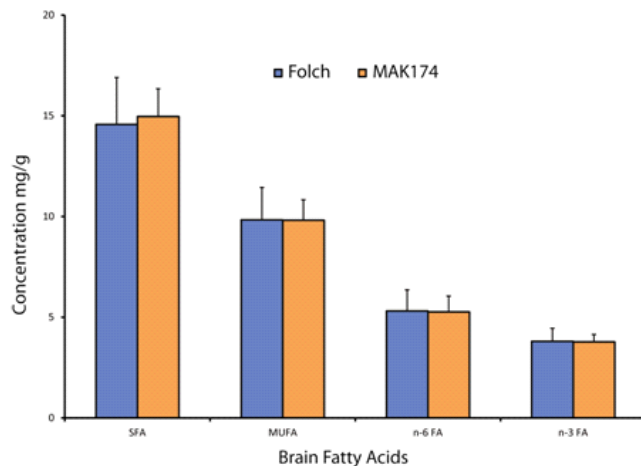
$$\frac{\text{Amount of internal standard (mg)} \times \text{Area of sample Lipid peak}}{\text{Area of internal standard} \times \text{Weight of tissue (g)}}$$

Amount of internal standard = 0.15 mg per sample when using the Extraction Solvent, which contains an internal standard.

### 2. Data comparing Folch standard method to MAK174 Kit method

#### Figure 1.

Rat brain fatty acid concentrations (mg/g)



Lipids were extracted from rat brain with the Folch or MAK174 kit method, transesterified, and quantified with GC-FID.

SFA = saturated fatty acids

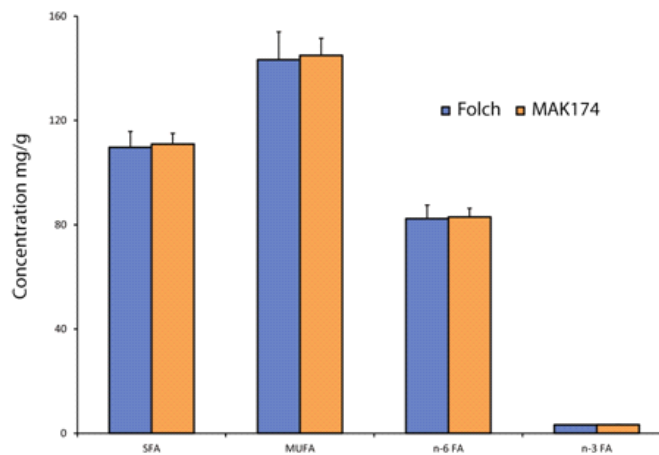
MUFA = monounsaturated fatty acids

n-6 FA = omega-6 polyunsaturated fatty acids

n-3 FA = omega-3 polyunsaturated fatty acids

#### Figure 2.

Powdered egg fatty acid concentrations



#### Egg Fatty Acids

Lipids were extracted with the Folch or MAK174 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

#### 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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