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Product Information

Fatty Acid Methylation Kit
Manufactured by Nacalai Tesque, Inc.

Catalog Number **MAK224**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description
Methyl esterification of fatty acids is commonly done prior to gas chromatography analysis to prevent peak tailing and to increase sample volatility. However, the conventional esterification procedure requires specialized equipment and advanced technical skill.

Quantitative capability of the conventional method is questionable due to the high heating requirement. The high temperature causes the degradation of unstable fatty acids (polyunsaturated and cyclopropane fatty acids) and the evaporation of short-chain fatty acid akyl esters.

By using the Fatty Acid Methylation Kit that employs a new proprietary reaction technique, followed by the Fatty Acid Methyl Ester Purification Kit (Catalog Number MAK225), the fatty acid methyl esterification process is greatly simplified.

Key Features:
- This kit is for methyl esterification of fatty acid samples prior to GC analysis.
- The methyl esterification can be performed safely and easily without excessive heating.
- Reaction is conducted at 37 °C.
- Detects long-chain and short-chain fatty acids.
- Applicable for free fatty acids and glycerolipids, such as triglycerides, phospholipids, glycolipids, and sterol esters.

Workflow:
Sample
↓ Methylation Reagent A – 0.5 mL
↓ Methylation Reagent B – 0.5 mL, 37 °C, 1 hour
↓ Methylation Reagent C – 0.5 mL, 37 °C, 20 minutes
↓ Isolation Reagent – 1.0 mL
Sample
↓ Deionized Water – 1.0 mL
Sample
Purification with Fatty Acid Methyl Ester
↓ Purification Kit MAK225 - If using a packed column, this step is not required.
GC Analysis

Components
The kit is sufficient for 100 reactions.

Methylation Reagent A Catalog Number MAK224A	50 mL
Methylation Reagent B Catalog Number MAK224B	50 mL
Methylation Reagent C Catalog Number MAK224C	50 mL
Isolation Reagent Catalog Number MAK224D	250 mL

Reagents and Equipment Required but Not Provided.
- Hermetically-closable test tube
- Heating block
- Vortex mixer
- Sample
- Acetic acid (only for glycerolipid analysis)

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.

Preparation Instructions
Preparation of various starting material:
E. coli or Yeast
Centrifuge cell culture medium of *E. coli* or Yeast in a centrifuging tube, and then freeze-dry ~20 mg of the pellet. An alternate method for an *E. coli* sample is to dry in a vacuum desiccator for 1-2 hours.

Blood
Preparation of BHT-treated filter paper - Soak filter paper in acetone containing 0.05% BHT (antioxidant) for several minutes, and then repeat this process. (Prepare a new 0.05% BHT solution for the second immersing.) Let the paper air dry at room temperature, then put it in a vacuum desiccator for 30 minutes or in a desiccator overnight. Square paper (1.5 cm on a side) is suitable for methylation application.
Note: Thicker filter papers, e.g., blood collecting filter papers, have low methylation efficiency.

Apply 0.04 mL of heparin-treated blood to BHT-treated filter paper and dry in a vacuum desiccator for 30 minutes, or let it air dry for more than 2 hours. To get complete methylation, spread the heparin-treated blood on filter paper evenly.

Rat Liver
Lyophilize 15 mg of rat liver.
Note: Drying rat liver in a vacuum desiccator decreased methylation efficiency.

Edible Oil
Less than 4 mg of edible oil is suitable for the methylation application.

Soybean Flour
Less than 20 mg of soybean flour is suitable for the methylation application.

Fish
Put 200 mg of fish meat, e.g., Japanese horse mackerel, into a test tube, and add 2 mL of Isolation Solution. Then grind the fish meat with a glass rod.

After vortexing, place 0.5 mL of supernatant in a new test tube, and then dry it in a rotary evaporator, vacuum desiccator, or N_2 gas.

Storage/Stability
The kit is shipped at ambient temperature. Store all components at room temperature upon receiving.

Methylation B Reagent is sensitive to air and deteriorates quickly; use it as quickly as possible.

Procedure
All samples and standards should be run in duplicate.

A. Protocol for General Fatty Acids
1. Put dried sample into a hermetically-closable test tube.
2. Add 0.5 mL of Methylation Reagent A to the test tube.
3. Add 0.5 mL of Methylation Reagent B to the test tube (Mixing Methylation Reagents A and B beforehand and adding together to the test tube is also acceptable, but use freshly mixed solution).
4. Close the cap tightly and incubate the test tube at 37 °C for an hour or at room temperature overnight (for methylation of glycerolipids and sterol esters). If the sample does not contain sterol esters or in a very small amount, the incubation time can be shortened to 5 minutes at 37 °C or 20 minutes at room temperature.
5. Add 0.5 mL of Methylation Reagent C.
6. Close the cap tightly and incubate the test tube for 20 minutes at 37 °C (for methylation of free fatty acids).
7. Add 1.0 mL of Isolation Reagent and vortex.
8. After observing the separation of two layers, transfer the upper layer to a new tube with a pipette, avoid picking up the milky layer. If the volume of the upper layer is low, centrifugation is required.
9. Add 1.0 mL of deionized water to the test tube containing the upper layer and mix.
10. Transfer the upper layer to a new test tube.
11. If the GC analysis is done with capillary columns, further purification with the Fatty Acid Methyl Ester Purification Kit (Catalog Number MAK225) is required. If packed columns are used, no further purification step is required (skip step 12).

12. Purification step with Fatty Acid Methyl Ester Purification Kit (Catalog Number MAK225). All steps are done under gravity fall condition.
    a. Add 3 mL of Conditioning Solution to a SPE Cartridge Column for conditioning.
    b. Add the methylated sample from the Fatty Acid Methylation Kit (Catalog Number MAK224) to the SPE Cartridge Column.
    c. Add 3 mL of Washing Solution to the SPE Cartridge Column for washing.
    d. Add 3 mL of Eluting Solution and collect eluted liquid containing fatty acid methyl esters from the SPE Cartridge Column.
13. Inject the eluted liquid from step 12 or the supernatant from step 10 into a GC column. If the sample concentration is too low, reduce the sample volume with vacuum desiccator, N_2 gas, or rotary evaporator. Dried sample can be dissolved into a small amount of Isolation Reagent and then injected into a GC column.

B. Protocol for Glycerolipids

If a sample does not contain free fatty acids and sterol esters (or in low abundance), Procedure B can be used. It is a simplified method without the use of Methylation Reagents A and C.

1. Put dried sample into a hermetically-closable test tube tube.
2. Add 2.0 mL of Isolation Reagent to the test tube.
3. Add 0.2 mL of Methylation B to the test tube.
4. Close a cap tightly, incubate until the temperature rises to 30-37 °C, and then vortex for 2 minutes, or ultrasonicate the test tube in a bath at 30-37 °C for 2-3 minutes. (For high-throughput analysis, vortexing or ultrasonication of the entire test tube stand is acceptable. Please note ultrasonic waves may not be uniform across the water bath depending on the location.)
5. Add 0.02 mL of acetic acid to lower the pH, and add 2 mL of deionized water. Mix well.
6. After observing the separation of two layers, transfer the upper layer to a new tube with a pipette, avoid picking up the milky layer. If the volume of the upper layer is low, centrifugation is required.
7. For GC analysis with a capillary column, further purification by the Fatty Acid Methyl Ester Purification Kit (Catalog Number MAK225) is required (refer to step 8). For analysis by a packed column, no further purification step is required (skip step 8).
8. Purification by the Fatty Acid Methyl Ester Purification Kit (Catalog Number MAK225). All steps are under gravity fall conditions.
    a. Add 3 mL of Conditioning Solution to a SPE Cartridge Column.
    b. Add the methylated sample from the Fatty Acid Methylation Kit (Catalog Number MAK224) to the SPE Cartridge Column.
    c. Add 3 mL of Washing Solution to the SPE Cartridge Column.
    d. Add 3 mL of Eluting Solution to the SPE Cartridge Column and collect eluted liquid containing fatty acid methyl esters.
9. Inject the eluted liquid from step 8 or the supernatant from step 6 into a GC column. If the sample concentration is too low, reduce the sample volume with a vacuum desiccator, N_2 gas, or rotary evaporator. Dried sample can be dissolved into a small amount of Isolation Reagent and then injected into a GC column.

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