Contact us
Email : info.intl@nacalai.co.jp
TEL : +81-75-251-1730
FAX : +81-75-251-1763

06482E_1411_5

Product No. 06482

Fatty Acid Methylation Kit (100 TESTS)

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
- Applicable for free fatty acids, glycolipids and sterol esters
- Not applicable for sphingolipids

Components

Reagents	Volume	Quantity
Methylation Reagent A	50 ml	1
Methylation Reagent B*1	50 ml	1
Methylation Reagent C*1	50 ml	1
Isolation Reagent	250 ml	1

*1 Extreme care must be taken when handling Methylation Reagent B (strong alkaline solution), and Methylation Reagent C (strong acidic solution).

Required reagents and equipments

- Hermetically-closable test tube such as screw-top test tube, φ16.5 mm x 125 mm
- Heat block to 37 ˚C
- Pippetes
- Vortex mixer
- Target sample
- Acetic acid (only for glycolipids analysis as described in the Procedure 2)

Protocol 1, all fatty acid analysis

1. Put dried sample*2 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 Reagent A and B beforehand and adding it to the test tube is also fine, but please 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 the methylation of glycolipids and sterol esters). If the sample does not contain sterol esters or in a very small, amount, the incubation time can be shorten to 5 minutes at 37 °C or 20 min at room temperature.
5. Add 0.5 ml of Methylation Reagent C.
6. Close the cap tightly and incubate the test tube for 20 min at 37°C. (For the methylation of free fatty acids).
7. Add 1.0 ml of Isolation Reagent and vortex.
8. After seeing the presence of two layers, transfer supernatant to a new test tube with a pipette, avoid picking up the milky layer. If the amount of the supernatant is thin, centrifugation is required.
9. Add 1 ml of deionized water to the test tube containing the supernatant and mix it up for cleaning.
10. Transfer the supernatant to a new test tube.
11. If the GC analysis is done with capillary columns, further purification with Fatty Acid Methyl Ester Purification Kit (Product No. 06483) 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 (Product No. 06483). All steps are done under gravity fall condition.
    12-1. Add 3 ml of Conditioning Solution to a SPE Cartridge Column for conditioning
    12-2. Add the sample already methylated with Fatty Acid Methylation Kit to the SPE Cartridge Column
    12-3. Add 3 ml of Washing Solution to the SPE Cartridge Column for washing
    12-4. Add 3 ml of Eluting Solution and collect eluted liquid contained 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, N2 gas or rotary evaporator. Dried sample can be dissolved into a small amount of Isolation Reagents and then inject into GC columns.

Manufactured by NACALAI TESQUE, INC.
498 Higashitamaya-cho, Nijo Karasuma,
Nakagyo-ku, Kyoto 604-0855, Japan

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*2 Sample Preparation

Starting Material	Procedure
E. coli or Yeast	Centrifuge cell culture medium of E. Coli or Yeast in a centrifuging tube, and then freeze-dry about 20 mg of the pellet. An alternate method for the E. coli sample is to dry in a vacuum desiccator for 1-2 hours.
Blood	Apply 0.04 ml of heparin-treated blood to antioxidant agent BHT-treated filter paper and dry it in a vacuum desiccator for 30 min. or let it dry naturally for more than 2 hours. To get complete methylation, spread the heparin-treated blood on filter paper widely. [How to make antioxidant agent BHT-treated filter paper] Soak filter paper such as Whatman 3M or ADVANTECH No. 2 in acetone containing 0.05% BHT for several minutes and then repeat this process. (Prepare an alternative new solvent for the second immersing). Let it dry naturally at room temperature, then put it in a vacuum desiccator for 30 min. or in a desiccator overnight. The paper with a square 1.5 cm on a side is suitable for methylation application. Please note that thicker filter papers, e.g. blood collecting filter papers, have low methylation efficiency.
Rat Liver	Lyophilization of 15 mg of rat liver. Please note that drying rat liver in a vacuum desiccator decreases 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 mesh the fish meat with a glass rod. After vortexing, take 0.5 ml of supernatant to a new test tube, and then dry it in a rotary evaporator, vacuum desiccator, or N_2 gas.

Protocol 2, glycolipid analysis

If a sample does not contain free fatty acid and sterol esters (or in a small amount), Procedure 2 can be used. It is a simplified method without using of Methylation Reagents A and C.

1. Put dried sample*3 into a Hermetically-closable test tube.
2. Add 2.0 ml of Isolation Reagents to the test tube.
3. Add 0.2 ml of Methylation Reagent B to the test tube.
4. Close a cap tightly and incubate it until become to 30-37°C, and then vortex for 2 min. or ultrasonicate the test tube in a bath at 30-37°C for 2-3 min. (For high-throughput analysis, vortexing or ultrasonication the whole test tube stand is acceptable. Please note that 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 seeing the presence of two layers, transfer the supernatant to a new test tube with a pipette, avoiding picking up the milky layer. If the supernatant layer is too thin, centrifugation is required.
7. For GC analysis with a capillary column, further purification by the Fatty Acid Methyl Ester Purification Kit (Product No. 06483) is required (refer to step 8). For analysis by a packed column, no further purification step is required (skip step 8.)
8. Purification step with the Fatty Acid Methyl Ester Purification Kit (Product No. 06483). All steps are under gravity fall condition.
   8-1. Add 3 ml of Conditioning Solution to a SPE Cartridge Column
   8-2. Add a sample methylated with Fatty Acid Methylation Kit to the SPE cartridge Column
   8-3. Add 3 ml of Washing Solution to the SPE Cartridge Column
   8-4. 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 vacuum desiccator, N2 gas or rotary evaporator. Dried sample can be dissolved into a small amount of Isolation Reagents and then inject into GC columns.

*3 Please follow the Procedure for Sample Preparation.

Caution

- Wear protective glasses and gloves.
- Wash with copious amount of water if your skin is exposed to reagents
- Methylation B Reagent is sensitive to air and deteriorates quickly, use it as soon as possible.

Storage

Room Temperature

Packing

100 TESTS (Product No.06482-04)

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