

Methyl esterification kit for fatty acids analysis

Fatty Acid Methylation Kits

Below are two methods for efficiently preparing fatty acid samples for GC analysis. Neither method requires high temperatures, unlike the conventional method, so methylation can be done safely and easily.

	Fatty Acid Methylation Kit ▶ See P.2	Fatty Acid Methylation Kit for Glycerides NEW ▶ See p.4
Targeted fatty acids	<ul style="list-style-type: none"> • Glycerides (glycerolipids, such as triglycerides, diglycerides, monoglycerides and lecithin) • Free fatty acids • Sterol esters 	<ul style="list-style-type: none"> • Glycerides (glycerolipids, such as triglycerides, diglycerides, monoglycerides and lecithin) • Glycerides containing short-chain fatty acids
Targeted samples	Blood, yeast, liver, cooking oil, soybean powder, fish oils	Cooking oil, fish oils
Procedure		
	<div style="border: 1px solid black; padding: 10px; width: fit-content; margin: auto;"> <p>Conventional Method</p> </div>	
	<ul style="list-style-type: none"> • React at 37°C • Simple procedure 	<ul style="list-style-type: none"> • React at room temp. • Fast and simple procedure
Reaction time	1.5 hrs	Less than 1 min
Reaction temperature	37°C	Room temp.
Quantitative analysis*	Very good	Good
Experimental precision	Very good	Good

* This difference is due to a small amount of free fatty acids in cooking oil.

Fatty Acid Methylation Kit

Features

- ▶ For the analysis of volatile free fatty acids, glycerolipids and sterol esters
- ▶ React at 37 °C
- ▶ Conduct methyl esterification safely and easily
- ▶ Detects not only long-chain, but also short-chain fatty acids



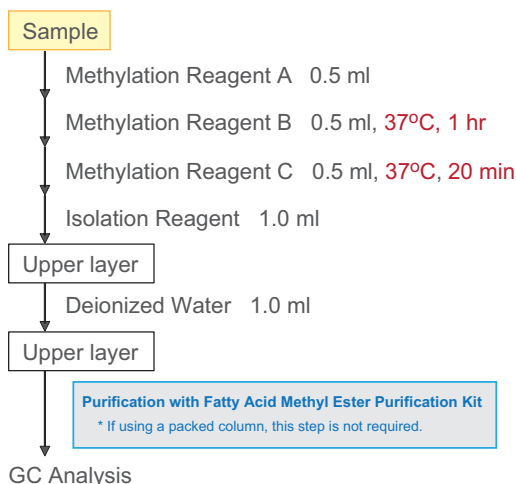
Targeted fatty acids

- Free fatty acids
- Glycerolipids (such as triglycerides), phospholipids and glycolipids
- Sterol esters

Please note that this method is not suitable for sphingolipids.

Procedure

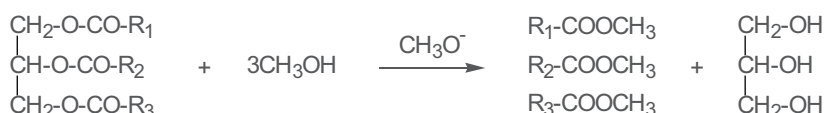
Fatty Acid Methylation Kit



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.
4. Close the cap tightly and incubate the test tube at 37°C for an hour or at room temperature overnight.
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.
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.
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 is required. (All steps are done by gravity flow.)
12. Analyze with GC.

Reaction Mechanism

Reaction with Methylation Reagent B

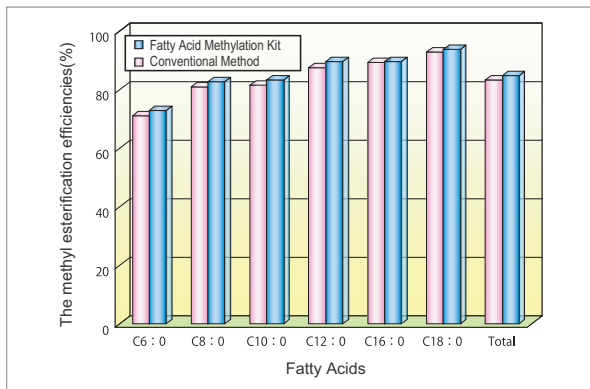
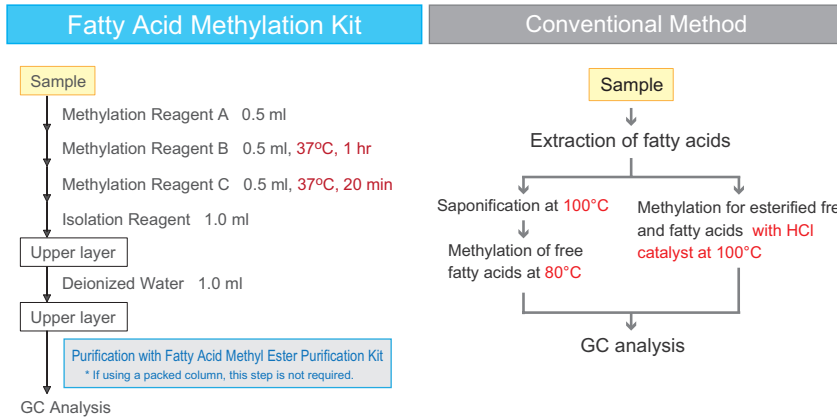


Reaction with Methylation Reagent C



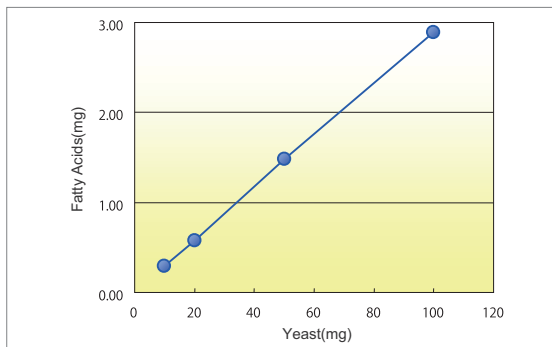
Comparison with Conventional Method

The methyl esterification efficiencies of the Fatty Acid Methylation Kit and the conventional method are about the same, independent of chain length. The 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 alkyl esters.



Data courtesy of GEKKEIKAN

Quantitative Analysis



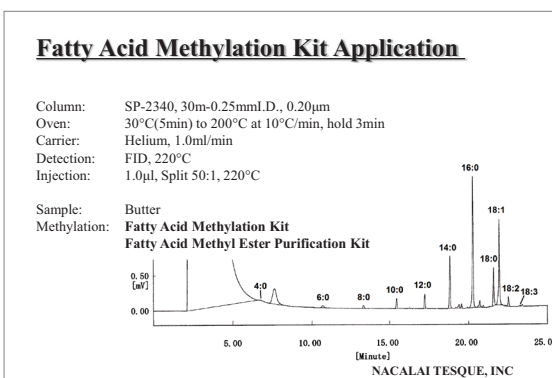
The Fatty Acid Methylation Kit and Fatty Acid Methyl Ester Purification Kit offer wide dynamic range as shown by the results from dried yeast.

Data courtesy of GEKKEIKAN

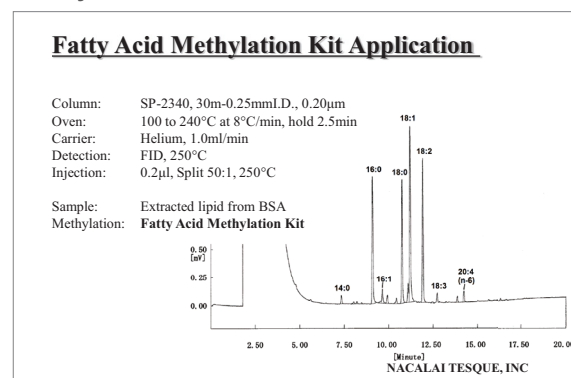
Applications

The lipid samples below were methylated using the Fatty Acid Methylation Kit and analyzed using GC.

• Butter



• Fatty Acids in BSA



Fatty Acid Methylation Kit for Glycerides

Features

- ▶ Methylate fatty acids from glycerides in your sample
- ▶ React at room temperature; suitable for volatile short-chain fatty acids
- ▶ Fast - stir for 3 seconds, let stand for 10 seconds
- ▶ Good for simple analysis
- ▶ Simple procedure

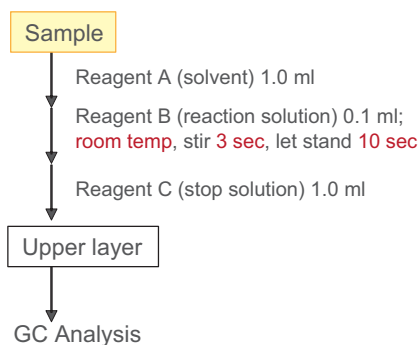


Targeted fatty acids

- Glycerides (glycerolipids, such as triglycerides, diglycerides, monoglycerides and lecithin)
 - * Cannot be used with free fatty acids, sterol esters or sphingolipids.
- This kit is suitable for measuring the fatty acid composition of glycerides. For other fatty acids (except sphingolipids), please use the regular Fatty Acid Methylation Kit (p. 2), which can also methylate free fatty acids and sterol esters.

Procedure

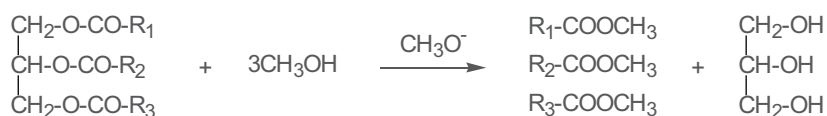
Fatty Acid Methylation Kit for Glycerides



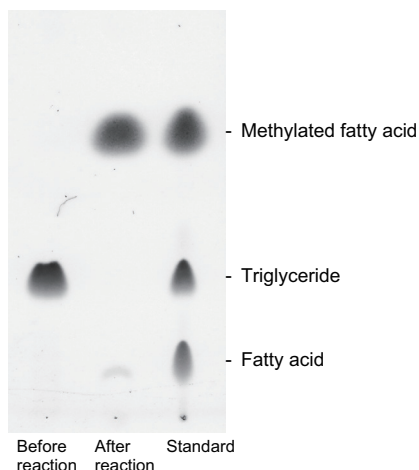
1. Place your glyceride sample (50 mg or less) in a suitable container.
 2. Add 1.0 ml of reagent A (solvent), and dissolve the sample.
 3. Add 0.1 ml of reagent B (reaction solution), and stir with a vortex mixer for 3 seconds.
 4. Let the mixture stand for about 10 seconds, then add 1.0 ml of reagent C (stop solution). Stir with a vortex mixer for 5-10 seconds.
 5. Let stand until two layers form. Move the upper layer to a different container, such as an autosampler vial.
 6. Analyze with GC.
- * This kit does not use the Fatty Acid Methyl Ester Purification Kit (p. 2).

Reaction Mechanism

Methylation is done with a transesterification (methanolysis) reaction between the glyceride and methanol.



Reaction Completion



Reaction was confirmed by methylating 10 of a triglyceride (triolein) using the **Fatty Acid Methylation Kit for Glycerides** and performing TLC using a silica gel plate.

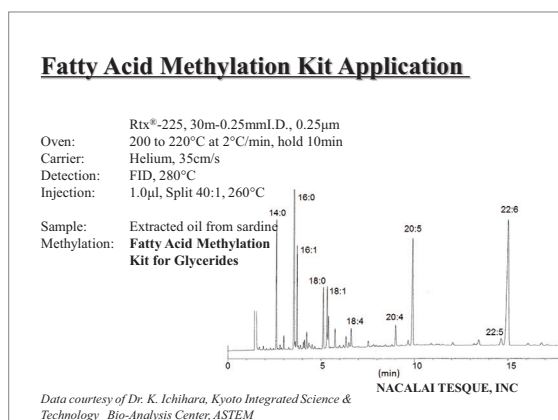
- Developing solution: Hexane / t-butyl methyl ether / acetic acid = 92 / 8 / 0.4
- Coloring: Sprayed with 50% sulfuric acid, then heated at 137°C for 15 minutes

(Results)

The triglyceride was successfully methylated.

Applications

• Fatty acid analysis of oil extracted from sardines



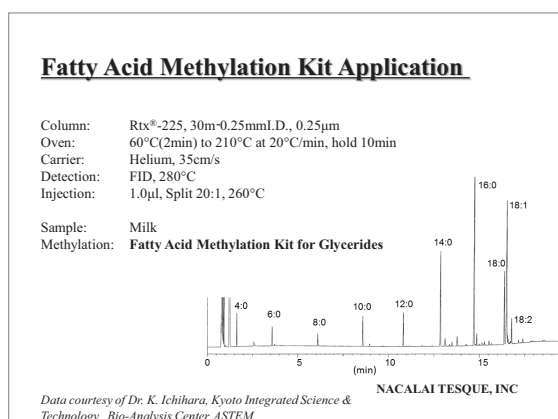
(Sample preparation)

1. Using a mortar and pestle, about 10 g of a small sardine was extracted with 30 ml of acetone, then 20 ml of hexane. After extraction, the mixture was filtered.
2. 30 ml of water was added to the filtrate and the solution was mixed.
3. The hexane layer was collected and evaporated in a rotary evaporator.
4. From the resulting oil, about 20 mg was collected and methylated using the Fatty Acid Methylation Kit for Glycerides.

(Results)

In addition to saturated fatty acids, the sample contained large amounts of unsaturated fatty acids, such as DHA and EPA.

• Analysis of fatty acids in milk



(Sample preparation)

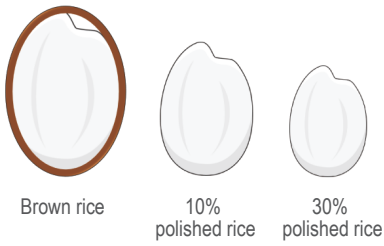
1. 0.5 ml of milk was combined with 2 ml of reagent A from the Fatty Acid Methylation Kit for Glycerides.
2. 1 ml of the organic layer was methylated with the Fatty Acid Methylation Kit for Glycerides.

(Results)

Methylation using the Fatty Acid Methylation Kit for Glycerides can be done quickly at room temperature, so volatile short-chain fatty acids, such as methyl butyrate (4:0) and methyl hexanoate, can be analyzed.

Applications (continued)

• Fatty acid analysis of rice (Nipponbare) for producing sake (effect of different degrees of polishing)

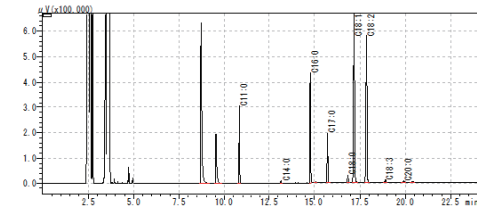


(Sample preparation)

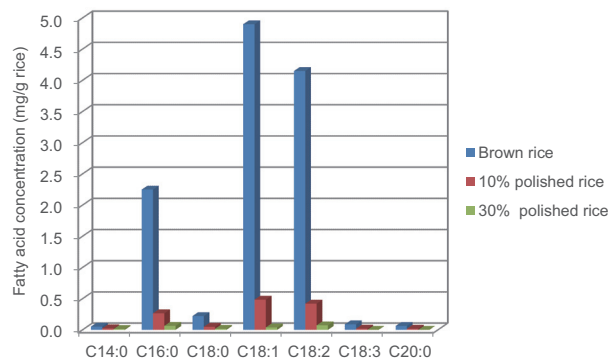
1. The samples (100 mg each of brown rice, 10% polished rice and 30% polished rice) were crushed.
2. 1 ml of acetone was added to the crushed samples and stirred with a vortex mixer. The acetone solution was collected.
3. 1 ml of hexane was added to the residues, stirred with a vortex mixer, collected and mixed with the acetone solutions.
4. 1 ml of ultra-pure water was added to the mixtures obtained in (3), and the mixtures were stirred lightly.
5. The mixtures obtained in (4) were centrifuged at 2000 rpm for 3 minutes, and the upper hexane layers were collected.
6. The hexane solutions were evaporated, leaving an oil sample.
7. Half of the oil (equivalent to 50 mg of rice) was methylated using the Fatty Acid Methylation Kit for Glycerides.

Fatty Acid Methylation Kit Application

Column: Stabilwax, 30m-0.53mmI.D., 0.25 μ m
 Oven: 50°C(1min) to 230°C at 15°C/min, hold 8min
 Carrier: Helium, 2.95ml/min
 Detection: FID, 250°C
 Injection: 1.0 μ l, Split 3:1, 250°
 Sample: Extracted lipid from unpolished rice



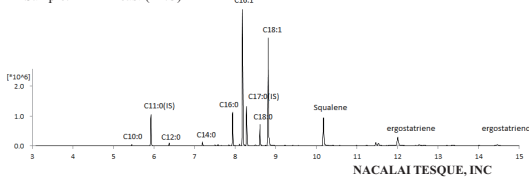
GC Assay



• Fatty acid analysis of refined sake yeasts

Fatty Acid Methylation Kit Application

Column: SP-2380, 30m-0.25mmI.D., 0.20 μ m
 Oven: 70°C(1min) to 230°C at 20°C/min, hold 7min
 Carrier: Helium, 0.6ml/min
 Detection: MSD, 230°C
 Injection: 2.0 μ l, Split 20:1, 250°C
 Sample: Yeast (1103)



Data courtesy of Gekkeikan Sake Company, Ltd.

(Sample)

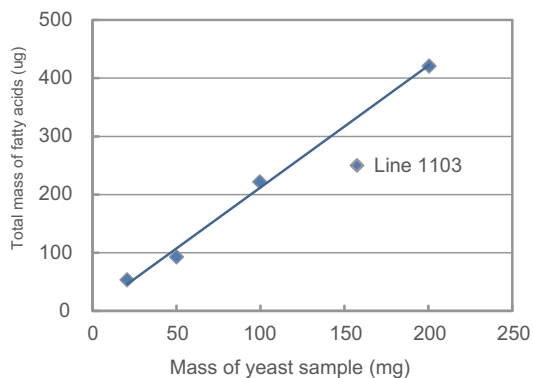
Yeast - parent cell line 1103

Yeast - mutant cell line 0101 (cerulenin-resistant)

(Sample preparation)

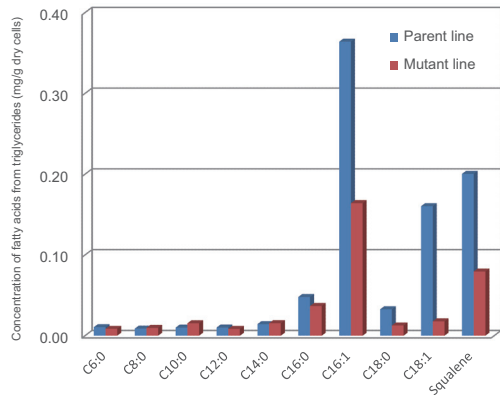
1. Yeast incubation (YPD, 30°C, approx. 24 hours)
2. After harvesting, the cultures were washed twice with distilled water.
3. The bacteria were lyophilized overnight.
4. Lipids were extracted with acetone and hexane.
5. The sample was methylated using the Fatty Acid Methylation Kit for Glycerides.

Quantification



Data courtesy of Gakkeikan Sake Company, Ltd.

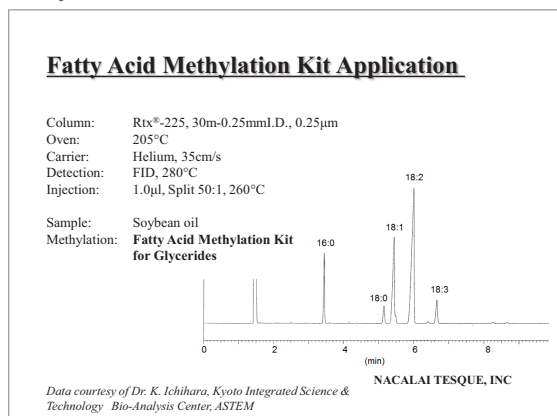
Composition of fatty acids in yeast cells



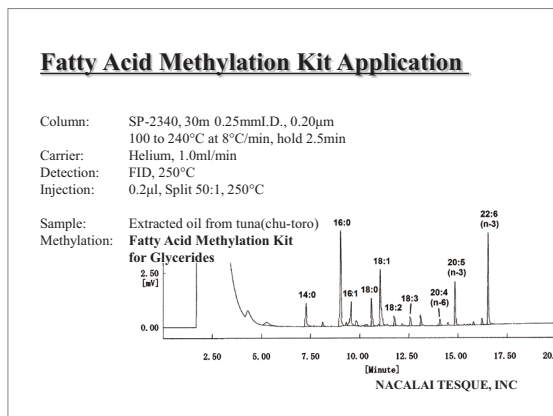
Applications (continued)

The lipid samples below were methylated using the Fatty Acid Methylation Kit for Glycerides and analyzed using GC.

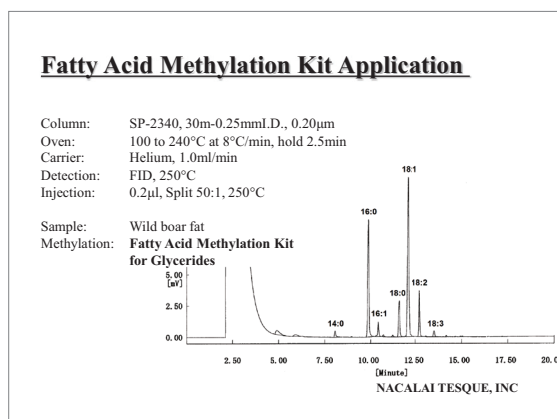
● Soybean oil



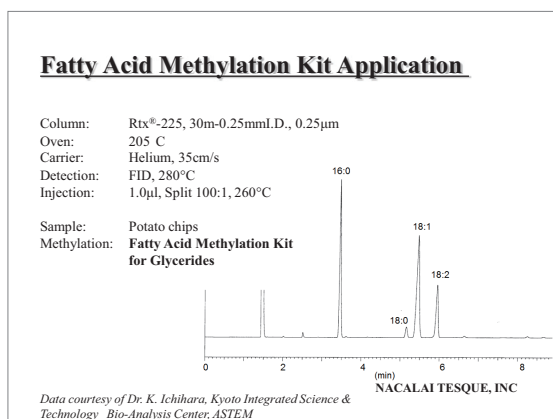
● Extracted oil from tuna



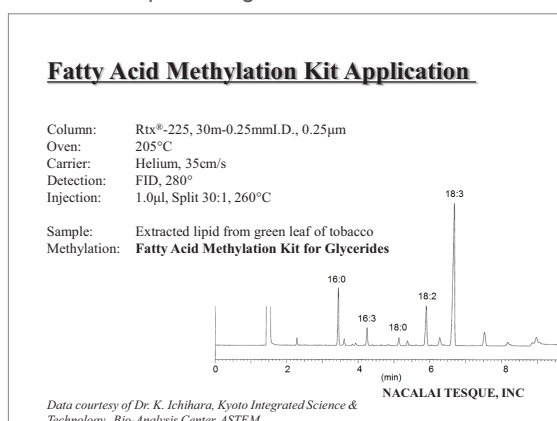
● Wild boar fat



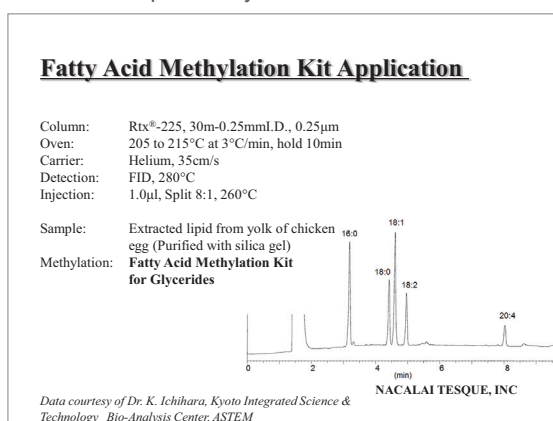
● Potato chips



● Extracted lipid from green leaf of tobacco



● Extracted lipid from yolk of chicken



Main components: Galactolipids (glycolipids), phospholipids

Main components: Phosphatidylcholines, phosphatidylethanolamines

For more Application, Please visit our website at http://www.nacalai.co.jp/global/cosmosil/related/Methylation_Kit.html
 (Over 50 Application available.)

Kit Contents

Fatty Acid Methylation Kit (100 tests)

Product Name	PKG Size	QTY
Methylation Reagent A	50 ml	1
Methylation Reagent B	50 ml	1
Methylation Reagent C	50 ml	1
Isolation Reagent	250 ml	1



Fatty Acid Methyl Ester Purification Kit (50 tests)

Product Name	PKG Size	QTY
Conditioning Solution	200 ml	1
Washing Solution	200 ml	1
Eluting Solution	200 ml	1
SPE Cartridge Column	-	50 pcs



Fatty Acid Methylation Kit for glycerides (100 tests)

Product Name	PKG Size	QTY
Solution A (Solvent)	100 ml	1
Solution B (Reaction Solution)	10 ml	1
Solution C (Stop Solution)	100 ml	1



Ordering Information

Product Name	Grade	Storage	Product No.	PKG Size
Fatty Acid Methylation Kit (100 tests)	SP	RT	16962-04	100 tests
Fatty Acid Methyl Ester Purification Kit (50 tests)	SP	RT	16961-14	50 tests
Fatty Acid Methylation Kit for glycerides (100 tests)	SP	RT	13246-84	100 tests

These products are covered by a patent acquired by Gekkeikan Sake Co., Ltd. in collaboration with Dr. Ichihara of Kyoto Integrated Science & Technology Bio-Analysis Center, ASTEM. Nacalai Tesque manufactures and sells these products under a license agreement. [Patent no. 4942380 (Japan)]

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