This package insert follows the Pharmaceuticals, Medical devices and Other Therapeutic Products Act of Japan.

In Vitro Diagnostics
Marketing Approval No. 20300AMZ00140000

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This package insert must be read carefully prior to use.

Class II Biochemical Test Series（General）
(Classification No.: 80022002)
Nonesterified fatty acid assay kit
(Classification No.: 30174000)

Clinimate NEFA

General Precautions

1. This product is for in vitro diagnostic use, and must not be used for any other purposes.
2. Clinicians should make a comprehensive clinical decision based on assay results in conjunction with clinical symptoms and other examination results.
3. This product should be used only as directed in this package insert. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.
4. If the reagent accidentally comes in contact with eyes and/or mouth, rinse immediately with ample water as first aid, and consult the doctor if required.
5. Carefully read the operating instructions for each type of automated analyzers prior to using this product. Parameters for each type of analyzers are available, and can be requested from SEKISUI MEDICAL CO., LTD. if required.
6. Perform a quality control test prior to assay to ensure accuracy.

Description (Kit Components) **

Component: Ingredients
NEFA Enzyme Agent 1:
Acyl CoA synthetase (Pseudomonas origin)
4-Aminoantipyrine
Ascorbate oxidase
Coenzyme A
Adenosine triphosphate disodium
Magnesium chloride
NEFA Buffer Solution 1:
2-Amino-2-hydroxymethyl-1, 3-propanediol buffer
NEFA Enzyme Agent 2:
Acyl CoA oxidase (Arthrobacter origin)
Peroxidase
NEFA Buffer Solution 2:
N, N-bis (4-sulfobutyl)-m-toluidine disodium (DSBmT)
N-Ethyl maleimide

Intended Use

Measurement of free fatty acids in serum

Free fatty acids include oleic acid, palmitic acid, stearic acid, and linoleic acid. Most of these fatty acids are bound to albumin in the blood.
The serum level of free fatty acids is profoundly influenced by lipid metabolism, glucose metabolism, and endocrine function, and is increased in patients with diabetes, severe hepatopathy, and hyperthyroidism.

Assay Principle

1. Assay Principle

Free fatty acids in samples are converted to acyl CoA by the action of CoA synthetase (ACS) in the presence of coenzyme A (CoA) and adenosine triphosphate (ATP). Acyl CoA is oxidized by acyl CoA oxidase (ACO) to produce hydrogen peroxide. Hydrogen peroxide causes oxidative condensation of 4-aminoantipyrine and N,N,-bis (4-sulfobutyl)-m-toluidine (DSBmT) in the presence of peroxidase (POD) and form a complex with a red-purple color. The content of free fatty acids is determined by measuring the absorbance of this complex.

$$\text{Free fatty acids} + \text{CoA} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{ACS}} \text{Acyl CoA} + \text{AMP} + \text{Pyrophosphoric acid}$$

$$\text{AcylCoA} + O_2 \xrightarrow{\text{ACO}} \text{2,3-Trans-enoyl CoA} + H_2O_2$$

$$H_2O_2 + \text{DSBmT} + \text{4-Aminoantipyrine} \xrightarrow{\text{POD}} \text{Red-purple color}$$

2. Features

This reagent is stable after preparation.

Procedural Precautions

1. Properties of Samples and Sampling Methods

1) Samples
Serum may be used.
2) Storage of samples
The isolated serum should be tested on the same day.

2. Interfering substances

1) Assay results are found to be insensitive by bilirubin (up to 5 mg/dL) and ascorbic acid (up to 50 mg/dL).
2) Hemolysis samples may result in positive error. Perform re-measurement by another test method if the results obtained are extremely high compared with those of other related variables.

3. Others

1) Always use Anaserum NEFA standard solution for calibration.
2) Precautions for assay range
If the concentration of sample exceeds assay range, dilute the sample with saline and repeat the measurement.

Dosage/Administration (Assay Procedure) **

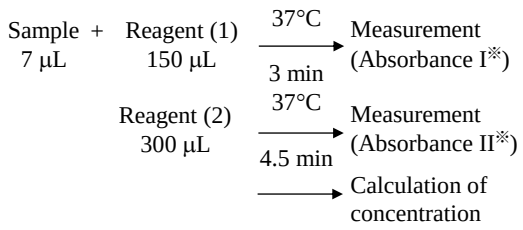
1. Preparation of reagents

Reagent (1): Dissolve NEFA Enzyme Agent 1 with NEFA Buffer Solution 1 before use.
Reagent (2): Disolve NEFA Enzyme Agent 2 with NEFA Buffer Solution 2 before use.
Reagents (1) and (2) are stable for 7 days after

preparation when stored at 2-10°C, under light shielding circumstance.

2. Assay Procedure

This product is compatible with various types of automated analyzer. An example of the assay procedure is indicated below.



※ Absorbance I and II: The difference in absorbance between 660 nm and 546 nm
Calibration material: Anaserum NEFA standard solution (Manufacture's assigned value)
Reagent blank: Purified water or saline

Assessment of Assay Results **

1. Reference standard range[4)]

140–850 μ Eq/L

2. There may be reactions or interfering reactions with non-target substances. If assay results appear to be unreliable, repeat the measurement (if necessary, after dilution) or try another analytical methods.

Performance

1. Sensitivity

1) Reagent blank: absorbance being equal to or lower than 0.06
2) Sensitivity: The absorbance is 0.24–0.30 per 1000 μ Eq/L of oleic acid.

2. Accuracy: 90–110% of the expected assay value

3. Within-run Reproducibility:

Coefficient of variation ≤ 3%

(Test methods used for 1–3. are in-house methods.)

4. Measurement Range[5)]: (On Hitachi 7170S automated analyzer)

10–3000 μ Eq/L

5. Correlation[5)]

Serum N=106 r=0.998 y=0.96x–20.11

Control method: Approved in vitro diagnostics (enzymatic method)

6. Standard Material

Sodium oleate (Manufacture's assigned value)

Precautions for Use or Handling **

1. Precautions for Handling (to Ensure Safety)

All samples used in the test should be handled as a material possibly infected with HIV, HBV, HCV, or other viruses. To prevent infection, use disposable gloves and avoid mouth pipetting during the test.

2. Precautions for use

1) This product should be stored as directed, without freezing. Freezing can deteriorate the reagents, which can produce inaccurate results. Therefore, avoid using the reagents which have been previously frozen.
2) Do not use expired reagents. Use of such reagents cannot guarantee the reliability of measurement values.
3) Do not replenish the reagents.
4) Do not perform the assay under direct sunlight

3. Precautions for Disposal

1) Before disposal, used samples and their containers must be immersed in sodium hypochlorite solution at a concentration of greater than 0.1% for longer than 1 hour or autoclaved at 121ºC for 20 minutes.
2) To prevent infections from spilled samples or solutions containing samples, wipe the spilled area thoroughly with disinfectants such as sodium hypochlorite solution at a concentration of greater than 0.1%.
3) The reagents and treated samples should be discarded as medical waste or industrial waste according to the waste disposal regulations.
4) The reagents should be disposed of in accordance with the Water Pollution Control act or related regulations.

4. Other precautions

Do not use the containers for other purposes.

Storage and Shelf Life **

1. Storage temperature: 2–10°C

2. Shelf life: 1 year from the date of manufacture
(The expiration date is printed on the outer package.)

Packaging **

Name			Package
Clinimate NEFA	(1)	NEFA Enzyme Agent 1	4×for 50mL
		NEFA Buffer Solution 1	4×50mL
	(2)	NEFA Enzyme Agent 2	4×for 100mL
		NEFA Buffer Solution 2	4×100mL

References **

1) Itakura H.: Clinical News 2 and 3, 1981.
2) Yamada H, Shimizu M, Tani Y.: Vitamin 54, 189, 1980.
3) Maehata E.: Med Tech, 8, 1029, 1980.
4) Kanai M. (supervising editor): Kanai's manual of clinical laboratory medicine. 34th ed. 516, Kanehara Shuppan, 2015.
5) In house data, SEKISUI MEDICAL CO., LTD.

Contact *

SEKISUI MEDICAL CO., LTD.
international@sekisui.com

Manufacturer **

SEKISUI MEDICAL CO., LTD.
1-3, Nihonbashi 2-chome, Chuo-ku, Tokyo, Japan