

NEFA FS*

Order Information

Cat. No. 1 5781 99 10 935 Kit size R1 2 x 20 mL + R2 1 x 10 mL

Kit for use in conjunction with DiaSys CE applications.

Intended Use

Diagnostic reagent for quantitative in vitro determination of non-esterified fatty acids (NEFA) in human serum or heparin plasma on automated photometric systems.

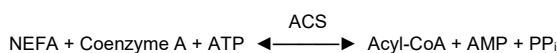
Summary

Non-esterified fatty acids serve the organism as source for metabolic energy and as substrate for cell membrane structures. Non-esterified fatty acids are released from adipose tissue by lipolysis [1]. The release is affected by diet and fluctuations of the insulin level. Pathological states as insulin resistance/diabetes type 2, adiposity and the metabolic syndrome are associated with increased concentrations of non-esterified fatty acids in blood and avail the development of cardiovascular diseases [2].

Method

Enzymatic endpoint method

Non-esterified fatty acids and coenzyme A react in the presence of acyl coenzyme A synthetase (ACS) to acylated coenzyme A. Subsequent oxidation of the acylated coenzyme A by acyl-CoA oxidase releases H₂O₂. H₂O₂ is converted to a colored product by the use of Trinder substances in the presence of peroxidase (POD).



At 546 nm the intensity of the red dye is directly proportional to the concentration of free fatty acids in the sample.

Reagents

Components and Concentrations

R1:	Goods buffer	pH 7.0	50 mmol/L
	Coenzyme A		0.4 g/L
	ATP		2 mmol/L
	Acyl CoA synthetase	(ACS)	0.4 kU/L
	MgCl ₂		2 mmol/L
R2:	Goods buffer	pH 7.0	50 mmol/L
	Acyl CoA oxidase	(ACOD)	30 kU/L
	Peroxidase	(POD)	45 kU/L

Storage and Stability

Reagents are stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C and contamination is avoided. Do not freeze and protect from light.

The open-vial stability of the reagent is 12 weeks until expiry date.

Warnings and Precautions

1. Components contained in NEFA FS are classified according to EC regulation 1272/2008 (CLP) as follows:



⚠ Reagent 1 and 2: Danger. Contains Alcohol, secondary, C12-C14, ethoxylated. H318 Causes serious eye damage. P280 Wear protective gloves/protective clothing/eye protection. P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310 Immediately call a POISON CENTER/doctor.

2. The reagents contain material of biological origin. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
3. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
4. In very rare cases, samples of patients with gammopathy might give falsified results [3].
5. In case of product malfunction or altered appearance that could affect the performance, contact the manufacturer.
6. Any serious incident related to the product must be reported to the manufacturer and the competent authority of the Member State where the user and/or patient is located.
7. Please refer to the safety data sheets (SDS) and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
8. For professional use only.

Waste Management

Refer to local legal requirements for chemical disposal regulations as stated in the relevant SDS to determine the safe disposal.

Warning: Handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Reagent Preparation

The reagents are ready to use.

Materials Required

General laboratory equipment

Specimen

Human serum or heparin plasma (fasting > 12 h) [4]

Only use suitable tubes or collection containers for specimen collection and preparation.

When using primary tubes, follow the manufacturer's instructions.

Samples from patients under heparin therapy are unsuitable for analysis. Measurement should be performed immediately after blood collection, since concentration of non-esterified fatty acids in serum increases due to lipolysis [5]. Store samples at -20°C, if direct measurement is not possible.

Only freeze once. Discard contaminated specimens.

Assay Procedure

Basic settings for BioMajesty® JCA-BM6010/C

Wavelength	545/596 nm
Temperature	37°C
Measurement	Endpoint
Sample/Calibrator	1.3 µL
Reagent 1	80 µL
Reagent 2	20 µL
Addition reagent 2	Cycle 19 (286 s)
Absorbance 1	Cycle 17/18 (231 s/244 s)
Absorbance 2	Cycle 41/42 (586 s/600 s)
Calibration	Linear

Calculation

With Calibrator

$$\text{NEFA [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Cal.}} \times \text{Conc. Cal. [mg/dL]}$$

Conversion Factor

$$\text{NEFA [mg/dL]} \times 0.0354 = \text{NEFA [mmol/L]}$$

Calibrators and Controls

DiaSys TruCal Lipid calibrator is recommended for calibration. Calibrator values have been made traceable to a primary standard material. NEFA Standard FS may be used alternatively for calibration. Use TruLab L Level 1 and Level 2 for internal quality control. All target values of the controls are traceable to DiaSys reagent/calibrator system. Quality control must be performed after calibration. Control intervals and limits have to be adapted to the individual requirements of each laboratory. Results must be within the defined ranges. Follow the relevant legal requirements and guidelines. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal Lipid	1 3570 99 10 045	3 x 2 mL
TruLab L Level 1	5 9020 99 10 065	3 x 3 mL
TruLab L Level 2	5 9030 99 10 065	3 x 3 mL
NEFA Standard FS	1 5780 99 10 065	3 x 3 mL

Performance Characteristics

Data evaluated on BioMajesty® JCA-BM6010/C

Measuring range up to 3 mmol/L, linearity is given within $\pm 5\%$. When values exceed this range, samples should be diluted 1 + 3 with NaCl solution (9 g/L) and the result multiplied by 4.

Limit of detection**	0.01 mmol/L
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Interference by	Interferences $\leq 10\%$ up to	Analyte concentration [mmol/L]
Ascorbic acid	30 mg/dL	0.623
Bilirubin (conjugated)	60 mg/dL	0.635
Bilirubin (unconjugated)	60 mg/dL	0.625
Hemolysis	100 mg/dL	0.677
Lipemia (triglycerides)	1100 mg/dL	0.810

For further information on interfering substances, refer to the literature [6,7].

Precision			
Repeatability (n=20)	Sample 1	Sample 2	Sample 3
Mean [mmol/L]	0.524	0.806	1.17
CV [%]	1.14	1.42	1.17
Between day (n=20)	Sample 1	Sample 2	Sample 3
Mean [mmol/L]	0.531	0.796	1.23
CV [%]	1.72	1.60	0.908

Method comparison (n=80)	
Test x	DiaSys NEFA FS (Hitachi 917)
Test y	DiaSys NEFA FS (BioMajesty® JCA-BM6010/C)
Slope	1.04
Intercept	0.006 mmol/L
Coefficient of correlation	0.999

** lowest measurable concentration which can be distinguished from zero; mean + 3 SD (n = 20) of an analyte free specimen.

Reference Range [8]

Women	0.1 – 0.45 mmol/L	2.8 – 12.7 mg/dL
Men	0.1 – 0.60 mmol/L	2.8 – 16.9 mg/dL

Plasma concentration of non-esterified fatty acids is subject to individual fluctuations and in particular increase after food intake.

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

1. Stich, V., & Berlan, M. (2004). Physiological regulation of NEFA availability: Lipolysis pathway. Proceedings of the Nutrition Society, 63(2), 369-374. doi:10.1079/PNS2004350
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4. Guder WG, Zatwa B et al. The quality of Diagnostic Samples. Darmstadt: GIT Verlag; 2001. page 28-9.
5. Stokol T, Nydam DV. Effect of Anticoagulant and Storage Conditions on Bovine Nonesterified Fatty Acid and β -Hydroxybutyrate Concentrations in Blood. J. Dairy Sci. 2005;88:3139-44.
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