NEFA FS*

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

Order information

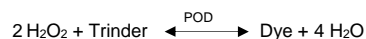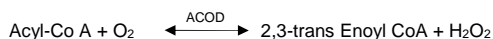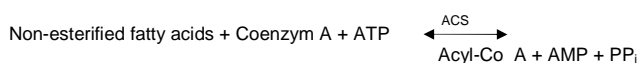| Cat. No.	Kit size				
1 5781 99 10 935	R1	2 x	20 mL +	R2 1 x	10 mL
1 5780 99 10 065		3 x	3 mL	Standard	

Summary [1,2]

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

Method

Enzymatic endpoint method

Principle

Non-esterified fatty acids and coenzyme A react in the presence of acyl coenzym A synthetase (ACS) to acylated coenzyme A. Acylated coenzyme A is oxidized by acyl coenzyme A oxidase under development of H_2O_2. H_2O_2 is converted to a coloured product by the use of Trinder substances in the presence of peroxidase (POD).

$$\text{Non-esterified fatty acids} + \text{Coenzym A} + \text{ATP} \xrightarrow{\text{ACS}} \text{Acyl-Co A} + \text{AMP} + \text{PP}_i$$

$$\text{Acyl-Co A} + O_2 \xrightarrow{\text{ACOD}} \text{2,3-trans Enoyl CoA} + H_2O_2$$

$$2\,H_2O_2 + \text{Trinder} \xrightarrow{\text{POD}} \text{Dye} + 4\,H_2O$$

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
Standard:			1 mmol/L

Storage Instructions and Reagent Stability

Reagents and standard are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C, protected from light and contamination is avoided. Do not freeze the reagents!

Warnings and Precautions

1. Reagent 1 and reagent 2: Danger. 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. Standard: Warning. H319 Causes serious eye irritation. 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. P337+P313 If eye irritation persists: Get medical advice/attention.
3. In very rare cases, samples of patients with gammopathy might give falsified results [6].
4. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
5. Please refer to the safety data sheets 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.
6. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The reagents and the standard are ready to use.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen [4,7]

Serum, heparin plasma or EDTA plasma (fasting > 12h)
Samples from patients under heparin therapy are unsuitable for analysis.
Effect the measurement immediately after blood collection because concentration of non-esterified fatty acids in serum increases due to lipolysis. Store samples at –20°C, if direct measurement is not possible.

Only freeze once!
Discard contaminated specimens!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	546 nm/600 nm (bichromatic)
Optical path	1 cm
Temperature	37°C
Measurement	Against reagent blank

	Blank	Sample/Standard
Sample/ Standard	-	20 µL
Dist. Water	20 µL	-
Reagent 1	1000 µL	1000 µL
Mix and incubate for 5 min. Read absorbance A1, then add:		
Reagent 2	250 µL	250 µL
Mix, incubate 10 min and read absorbance A2 within 20 min.		

ΔA = (A2 – A1) Sample/Standard

* fluid stable

Calculation

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

Conversion factor
Non-esterified fatty acids [mg/dL] x 0.0354 =
Non-esterified fatty acids [mmol/L]

Calibrator and Controls

For calibration, DiaSys TruCal Lipid or DiaSys NEFA Standard FS are recommended. The assigned values of the calibrator or standard are traceable to a primary standard material. DiaSys TruLab L controls should be assayed for internal quality control. 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

Performance characteristics

Measuring range
The test has been developed to determine non-esterified fatty acid concentrations up to 3 mmol/L. When values exceed this range samples should be diluted 1 + 3 with NaCl solution (9 g/L) and the result multiplied by 4.

Interferences
No interference is observed by ascorbic acid up to 30 mg/dL, bilirubin up to 60 mg/dL, lipemia up to 1000 mg/dL triglycerides and hemoglobin up to 200 mg/dL. For further information on interfering substances refer to Young DS [5].

Sensitivity/Limit of Detection
The lower limit of detection is 0.01 mmol/L.

Precision

Intra-assay n = 20	Mean [mmol/L]	SD [mmol/L]	CV [%]
Sample 1	0.29	0.00	1.07
Sample 2	0.49	0.01	1.05
Sample 3	0.88	0.01	0.98

Inter-assay n = 20	Mean [mmol/L]	SD [mmol/L]	CV [%]
Sample 1	0.61	0.01	1.15
Sample 2	1.02	0.01	1.07
Sample 3	1.38	0.02	1.10

Method Comparison
A comparison of DiaSys NEFA FS (y) with a commercially available test (x) using 114 samples gave following results:
y = 0.984 x + 0.045 mmol/L; r = 0.996

Reference range [3]

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 concentrations of non-esterified fatty acids are 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. Pilz S, Scharnagl H, Tiran B, et al. Free Fatty Acids Are Independently Associated with All-Cause and Cardiovascular Mortality in Subjects with Coronary Artery Disease. J Clin Endicrinol Metab 2006; 91: p. 2542-7.
2. Smith and Wilson. Free Fatty Acids and Atherosclerosis. J Clin Endocrinol Metab 2006; 91: p.2506-8.
3. Aufenanger J und Kattermann R. Klinisch-chemische Meßgröße: Freie Fettsäuren (FFS). In: Greiling H, Gressner AM: Lehrbuch der Klinischen Chemie und Pathobiochemie: Schattauer, 1995. p. 319-20.
4. Guder WG, Zatwa B et al. The quality of Diagnostic Samples. 1st ed. Darmstadt: Git Verlag, 2001: 28-9
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
6. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: Mechanisms, detection and prevention. Clin Chem Lab Med 2007; 45()): 1240–1243.
7. Stokol T and Nydam DV. Effect of Anticoagulant and Storage Conditions on Bovine Nonesterified Fatty Acid and ß-Hydroxybutyrate Concentrations in Blood. American Diary Science Association 2005. J. Diary Scl. 88: p. 3139-44.

Manufacturer

IVD CE DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany