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NEFA, ACOD-PAP

(en) English

Reagents with ATCS*

REF	Content
D07940	4 x 25 mL R1 + 1 x 25 mL R2
D07950	4 x 10 mL R1 + 1 x 10 mL R2
D77911	1 x 40 mL R1 + 1 x 10 mL R2
D0443917	4 x 50 mL R1 + 1 x 50 mL R2
DA0839	4 x 20 mL R1 + 1 x 20 mL R2
DT1039	4 x 20 mL R1 + 1 x 20 mL R2
DK0948	4 x 50 mL R1 + 1 x 50 mL R2
DE1839	1 x 50 mL R1 + 1 x 12.5 mL R2

* Advanced Turbidity Clearing System; minimizes turbidity caused by lipemia

For professional in vitro diagnostic use only.

INTENDED USE

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

DIAGNOSTIC SIGNIFICANCE

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.

TEST PRINCIPLE[1,2]

Non-esterified fatty acids and coenzyme A react in the presence of acyl coenzyme 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).

$$\text{NEFA + Coenzyme A + ATP} \xrightarrow{ACS} \text{Acyl-CoA + AMP + PPi}$$

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

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

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

REAGENT COMPOSITION

Components	Concentration	
Reagent 1		
Good´s 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
Reagent 2		
Good´s buffer, pH 7.0	50	mmol/L
Acyl CoA oxidase (ACOD)	30	kU/L
Peroxidase (POD)	45	kU/L

MATERIAL REQUIRED BUT NOT PROVIDED

- Calibrator or Standard, eg.:

REF	Name	Content
D13585SV	Diacal Lipids	1 x 2 mL
D07963SV	NEFA Standard	1 x 3 mL

- Controls, eg.:

REF	Name	Content	Description
D99486	Diacon Lipids	3 x 3 mL	lipid control normal
D99486SV	Diacon Lipids	1 x 3 mL	lipid control normal
D11487	Diacon Lipids High	3 x 3 mL	lipid control abnormal
D11487SV	Diacon Lipids High	1 x 3 mL	lipid control abnormal

- NaCl solution (9 g/L).
- Photometric device.
- General laboratory equipment.

REAGENT PREPARATION

Reagents and Standard are ready to use.

STORAGE AND STABILITY

Conditions:	Store at 2 – 8 °C. Protect from light. Close immediately after use. Avoid contamination. Do not freeze the reagents!
Stability:	91 days after first opening of the primary container
Calibration stability:	2 weeks
On-board stability:	4 weeks

WARNINGS AND PRECAUTIONS

1. Reagent 1 and 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.
Special labelling: Contains Alcohol, secondary, C12-C14, ethoxylated.

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.
6. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
7. In the event of an incident related to the device, report it to the manufacturer and your competent authority as required.
8. For professional use only!

SPECIMEN COLLECTION AND STORAGE[4,7]

Use 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.

Freeze only once!
Discard contaminated specimens!

STANDARD

(not included in the kit; has to be ordered separately)

Concentration:	1 mmol/L
Storage:	2 – 8 °C
Stability:	up to the indicated expiration date

Close immediately after use! Avoid contamination! Protect from light!

TEST PROCEDURE

Method:	Colorimetric, enzymatic, endpoint, increasing reaction
Wavelength:	546 nm / 600 nm (bichromatic)
Optical path:	1 cm
Temperature:	37 °C

Bring reagents and samples to room temperature.

Pipette into test tubes	Blank	Std./Cal.	Sample
Reagent 1	1000 µL	1000 µL	1000 µL
Sample	-	-	20 µL
Standard/Calibrator	-	20 µL	-
Distilled water	20 µL	-	-
Mix. Incubate for 5 min. at 37 °C. Read absorbance A1 against reagent blank, then add:			
Reagent 2	250 µL	250 µL	250 µL
Mix. Incubate for 10 min. at 37°C and read absorbance A2 against reagent blank within 20 minutes. ΔA = (A2 – A1)			

AUTOMATION

Applications for automated systems are available upon request.

INTERPRETATION OF RESULTS

Calculation

With Standard or Calibrator

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

Unit Conversion
NEFA [mg/dL] x 0.0354 = NEFA [mmol/L]

QUALITY CONTROL AND CALIBRATION

For internal quality control we recommend the DIALAB lipid control sera **Diacon Lipids** and **Diacon Lipids High**. Each laboratory should establish corrective action in case of deviations in control recovery.

Calibration
The assay requires the use of a NEFA Standard or Calibrator. We recommend the DIALAB **NEFA Standard** and the DIALAB lipid calibration plasma **Diacal Lipids**.

PERFORMANCE CHARACTERISTICS

Accuracy and precision
CV ≤ 1.07 % for within-run precision and CV ≤ 1.15 % for between-run precision.

Analytical sensitivity

Limit of detection: 0.01 mmol/L.

Linearity and measuring range

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

Analytical specificity

No interferences were observed for:

Ascorbic acid	≤ 30 mg/dL
Bilirubin	≤ 60 mg/dL
Hemoglobin	≤ 200 mg/dL
Triglycerides	≤ 1000 mg/dL

For further information on interfering substances refer to Young DS[5].

Clinical performance

A method comparison with an approved system using 114 samples gave the following results: y = 0.984 x + 0.045 mmol/L; r = 0.996.

Tests were performed on the following instrument: Hitachi 911.

TRACEABILITY

The assigned values of the calibrator or standard are traceable to a primary standard material.

EXPECTED VALUES[3]

	mg/dL	mmol/L
Women	2.8 – 12.7	0.10 – 0.45
Men	2.8 – 16.9	0.10 – 0.60

Plasma concentrations of non-esterified fatty acids are subject to individual fluctuations and in particular increased 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.

LIMITATIONS

- Eventual NEFA, ACOD-PAP carry-over to reagents Magnesium (Xylidyl blue) and Protein Total in Urine/CSF (Pyrogallol red). The actual carry-over depends on the analyser.

WASTE MANAGEMENT

Please refer to local legal requirements.

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.

USED SYMBOLS

Symbol	Description
Cont.	Content