CE IVD DIALAB

Liquid Reagents – ready to use

NEFA
(Non-Esterified Fatty Acids)

ACOD-PAP with ATCS*

2 Reagents

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

REF Cont.

D07940	**5 x 25 ml**	4 x 25 ml	Reagent 1
		1 x 25 ml	Reagent 2
D07950	**5 x 10 ml**	4 x 10 ml	Reagent 1
		1 x 10 ml	Reagent 2

Additionally offered:

D07963SV	1 x 3 ml	NEFA Standard	
D99486	3 x 3 ml	Lipid Control Normal	Diacon Lipids

TEST PARAMETERS

Method:	Colorimetric, Enzymatic
	Increasing Reaction, Endpoint
Wavelength:	546 nm / 600 nm (bichromatic)
Temperature:	37°C
Sample:	Serum, EDTA-plasma
Linearity:	up to 85 mg/dl (3 mmol/L)
Sensitivity:	The lower limit of detection is 0.28 mg/dl (0.01 mmol/L)

* **A**dvanced **T**urbidity **C**learing **S**ystem; minimzes turbidity caused by lipemia

REAGENT COMPOSITION

COMPONENTS	FINAL CONCENTRATION
Reagent 1:	
Goods buffer, pH 7.0	50 mmol/L
Coenzyme A	0.4 g/L
ATP	4 mmol/L
Acyl-CoA Synthetase (ACS)	0.4 kU/L
$MgCl_2$	2 mmol/L
Trinder coupling component	
Detergents and Stabilizers	
Reagent 2:	
Goods Buffer, pH 7.0	50 mmol/L
Acyl-CoA Oxidase (ACOD)	30 kU/L
Peroxidase (POD)	45 kU/L
Trinder coupling component	
Detergents and Stabilizers	

REAGENT PREPARATION

Substrate Start:
Reagents are ready for use.

Sample Start:
Not possible (sample blank).

REAGENT STABILITY AND STORAGE

Conditions:	protect from light avoid contamination do not freeze! close immediately after use
Storage:	at 2 – 8°C
Stability:	up to the expiration date

SAMPLE STABILITY AND STORAGE

Serum or 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.

Discard contaminated specimens.

STANDARD

(has to be ordered separately)

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

CLOSE IMMEDIATELY AFTER USE!

INTERFERING SUBSTANCES

no interference up to:

ascorbic acid	30 mg/dl
bilirubin	60g/dl
triglyceride	1000 mg/dl
hemoglobin	200 mg/dl

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

Substrate start

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

CALCULATION (light path 1 cm)

$$\text{NEFA (mg/dl)} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std/Cal}} \times \text{Conc. Std/Cal (mg/dl)}$$

UNIT CONVERSION

mg/dl x 0.0354 =mmol/L

REFERENCE RANGE [3]

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

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. For diagnostic purposes NEFA values should always be assessed in conjunction with the anamnesis, the clinical examination and other findings.

TEST 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).

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

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

2 H_2O_2 + Trinder $\xrightarrow{POD}$ 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.

PERFORMANCE CHARACTERISTICS

LINEARITY

The assay is linear up to 85 mg/dl (3 mmol/L).
Above this concentration, dilute the sample 1+3 with NaCl solution (9 g/L sodium chloride in water) and repeat the assay multiplying the result by 4.

PRECISION (at 37°C)

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 between Dialab NEFA (y) and a commercially available test (x) using 114 samples gave following results: y = 0.984 x + 0.045 mmol/L; r= 0.996.

QUALITY CONTROL

All control sera with NEFA values determined by this method can be used.
We recommend:

REF Cont.

D99486 3 x 3 ml **DIACON LIPIDS** Assayed Control Serum Normal

CALIBRATION

The assay requires the use of a NEFA Standard or Calibrator.
We recommend:

REF Cont.

D07963SV 1 x 3 ml **NEFA STANDARD**

AUTOMATION

Special adaptations for automated analyzers can be made on request.

WARNINGS AND PRECAUTIONS

Take the necessary precautions for the use of laboratory reagents.

WASTE MANAGEMENT

Please refer to local legal requirements.

REFERENCES

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;91:2542-7.
2. Smith and Wilson. Free Fatty Acids and Atherosclerosis. J Clin Endocrinol Metab 2006;91:2506-8
3. Kattermann R. Lipid- und Lipoproteinstoffwechsel. In: Greiling H, Gressner AM: Textbook Clinical Chemistry and Pathobiochemistry: Schattauer, 1987. p. 223-65

2°C 8°C IVD

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