respons®910

NEFA FS*

Diagnostic reagent for quantitative in vitro determination of non-esterified fatty acids (NEFA) in serum or plasma on DiaSys respons®910

Order Information

Cat. No. 1 5781 99 10 921
4 twin containers for 120 tests each

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).

Non-esterified fatty acids + Coenzym A + ATP $\xleftrightarrow{ACS}$ Acyl-Co A + AMP + PP_i

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

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

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

The reagents and the 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. DiaSys respons containers provide protection from light. Do not freeze 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 are ready to use. The bottles are placed directly into the reagent trays.

Specimen [1,7]

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. Discard contaminated specimens. Freeze only once.

Calibrators and Controls

For calibration, the DiaSys TruCal Lipid or DiaSys NEFA Standard FS is recommended. The assigned values of the calibrator or standard are traceable to a primary standard material. For internal quality control DiaSys TruLab L control should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
NEFA Standard FS	1 5780 99 10 065	3 x 3 mL
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 up to 3 mmol/L (84.7 mg/dL) NEFA (in case of higher concentrations re-measure samples after manual dilution with NaCl solution (9 g/L) or use rerun function).	
Limit of detection**	0.02 mmol/L (0.565 mg/dL) NEFA
On-board stability	21 days
Calibration stability	7 days

Interfering substance	**Interferences < 10%**	**NEFA [mmol/L]**
Ascorbate	up to 30 mg/dL	0.910
Hemoglobin	up to 120 mg/dL	0.600
	up to 180 mg/dL	0.960
Bilirubin, conjugated	up to 60 mg/dL	0.620
	up to 60 mg/dL	1.28
Bilirubin, unconjugated	up to 70 mg/dL	0.550
	up to 70 mg/dL	0.930
Lipemia (triglycerides)	up to 250 mg/dL	0.540
	up to 2000 mg/dL	0.890
For further information on interfering substances refer to Young DS [2].		

Precision			
Within run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mmol/L]	0.31	0.62	0.94
Coefficient of variation [%]	1.68	1.95	1.27
Between run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mmol/L]	0.27	0.40	1.45
Coefficient of variation [%]	3.75	2.81	1.50

Method comparison (n=150)	
Test x	DiaSys NEFA FS (Hitachi 917)
Test y	DiaSys NEFA FS (respons®910)
Slope	1.00
Intercept	0.00 mmol/L
Coefficient of correlation	0.999

** according to NCCLS document EP17-A, vol. 24, no. 34

Conversion factor

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

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 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.

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Literature

1. Guder WG, Zatwa B et al. The quality of Diagnostic Samples. 1st ed. Darmstadt: Git Verlag, 2001: 28-9.
2. 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.
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. 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.
5. Smith and Wilson. Free Fatty Acids and Atherosclerosis. J Clin Endocrinol Metab 2006; 91: p.2506-8.
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

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Application for serum and plasma samples

This application was set up and evaluated by DiaSys. It is based on the standard equipment at that time and does not apply to any equipment modifications undertaken by unqualified personnel.

Identification	
This method is usable for analysis:	Yes
Twin reaction:	No
Name:	NEFA
Shortcut:	
Reagent barcode reference:	048
Host reference:	048

Technic	
Type:	End point
First reagent:[µL]	180
Blank reagent	Yes
Sensitive to light	
Second reagent:[µL]	45
Blank reagent	No
Sensitive to light	
Main wavelength:[nm]	546
Secondary wavelength:[nm]	600
Polychromatic factor:	1.0000
1 st reading time [min:sec]	(04:24)
Last reading time [min:sec]	10:00
Reaction way:	Increasing
Linear Kinetics Substrate depletion: Absorbance li	
Linearity: Maximum deviation [%]	
Fixed Time Kinetics Substrate depletion: Absorbance limit	
Endpoint Stability: Largest remaining slope	
Prozone Limit [%]	

Reagents	
Decimals	
Units	

Sample	
Diluent	DIL A (NaCl)
Hemolysis:	
Agent [µL]	0 (no hemolysis)
Cleaner	
Sample [µL]	0
Technical limits	
Concentration technical limits-Lower	0.0200
Concentration technical limits-Upper	3.0000
SERUM	
Normal volume [µL]	3.0
Normal dilution (factor)	1
Below normal volume [µL]	
Below normal dilution (factor)	
Above normal volume [µL]	3.0
Above normal dilution (factor)	6
URIN	
Normal volume [µL]	3.0
Normal dilution (factor)	1
Below normal volume [µL]	
Below normal dilution (factor)	
Above normal volume [µL]	3.0
Above normal dilution (factor)	6
PLASMA	
Normal volume [µL]	3.0
Normal dilution (factor)	1
Below normal volume [µL]	
Below normal dilution (factor)	
Above normal volume [µL]	3.0
Above normal dilution (factor)	6
CSF	
Normal volume [µL]	3.0
Normal dilution (factor)	1
Below normal volume[µL]	
Below normal dilution (factor)	
Above normal volume [µL]	3.0
Above normal dilution (factor)	6
Whole blood	
Normal volume [µL]	3.0
Normal dilution (factor)	1
Below normal volume[µL]	
Below normal dilution (factor)	
Above normal volume [µL]	3.0
Above normal dilution (factor)	6

Results	
Decimals	2
Units	mmol/L
Correlation factor-Offset	0.0000
Correlation factor-Slope	1.0000

Range	
Gender	Male
Age	
SERUM	>=0.10 <=0.60
URINE	
PLASMA	>=0.1 0 <=0.60
CSF	
Whole blood	
Gender	Female
Age	
SERUM	>=0.10 <=0.45
URINE	
PLASMA	>=0.10 <=0.45
CSF	
Whole blood	

Contaminants
Please refer to r910 Carryover Pair Table

Calibrators details	
Calibrator list	**Concentration**
Cal. 1/Blank	0
Cal. 2	*
Cal. 3	
Cal. 4	
Cal. 5	
Cal. 6	
	Max delta abs.
Cal. 1	0.002
Cal. 2	0.005
Cal. 3	
Cal. 4	
Cal. 5	
Cal. 6	
Drift limit [%]	0.80

Calculations	
Model	X
Degree	1

* Enter calibrator value