NEFA FS*

Diagnostic reagent for quantitative in vitro determination of non-esterified fatty acids (NEFA) in serum or plasma on BioMajesty JCA-BM6010/C

Order Information

Cat. No. 1 5781 99 10 964
R1: 6 x 90 tests
R2: 6 x 90 tests

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 $\xrightarrow{ACS}$ Acyl-Co A + AMP + PP_i

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

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

At 545 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:	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
R2:	Good's buffer	pH 7.0	50 mmol/L
	Acyl CoA oxidase (ACOD)		30 kU/L
	Peroxidase (POD)		45 kU/L

Storage Instructions and Reagent Stability

The reagents 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 reagent!

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. In very rare cases, samples of patients with gammopathy might give falsified results [6].
3. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
4. 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.
5. 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.

Only freeze once! Discard contaminated specimens!

Calibrators and Controls

DiaSys TruCal Lipid or DiaSys NEFA Standard FS is recommended for calibration. 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 actions in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal Lipid	1 3570 99 10 045	3 x 2 mL
NEFA Standard FS	1 5780 99 10 065	3 x 3 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**	0.01 mmol/L (0.282 mg/dL) NEFA
On-board stability	12 weeks
Calibration stability	6 weeks

Interferences < 10% by
Ascorbate up to 30 mg/dL
Conjugated bilirubin up to 60 mg/dL
Unconjugated bilirubin up to 60 mg/dL
Hemoglobin up to 100 mg/dL
Lipemia (triglycerides) up to 1100 mg/dL
For further information on interfering substances refer to Young DS [5].

Precision			
Within run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mmol/L]	0.52	0.81	1.17
Mean [mg/dL]	14.8	22.8	33.0
Coefficient of variation [%]	1.14	1.42	1.17
Between run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mmol/L]	0.53	0.80	1.23
Mean [mg/dL]	15.0	22.5	34.6
Coefficient of variation [%]	1.72	1.60	0.91

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 (0.169 mg/dL)
Coefficient of correlation	0.999

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

Conversion factor

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

Reference Range [2]

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.

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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. 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.
3. 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.
4. Smith and Wilson. Free Fatty Acids and Atherosclerosis. J Clin Endocrinol Metab 2006; 91: p.2506-8.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Assocation 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(9): 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

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BioMajesty

NEFA FS

Chemistry code 10 578

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.

Analytical Conditions	
R1 volume	80
R2e volume	0
R2 volume	20
R1 diluent vol	0
R2e diluent vol	0
R2 diluent vol	0
Sample vol (S)	1.3
Sample vol (U)	1.3
Reagent 1 mix	weak
Reagent 2e mix	weak
Reagent 2 mix	weak
Reaction time	10

Sub-analy. Conditions	
Name	NEFA
Digits	2
M-wave L.	545
S-wave.L	596
Analy.mthd.	EPA
Calc.mthd.	STD
Qualit. judge	No

Analysis Test Condition Setting (M)		
Sample Type	**Serum**	**Urine**
Reac. sample vol.	1.3	1.3
Diluent method	No dil	No dil
Undil. sample vol.	0	0
Diluent volume	0	0
Diluent position	0	0

entered by user

Endpoint method	
Re.absorb (u)	9.999
Re. Absorb (d)	-9.999

Calculation Method Setting	
M-DET.P.I	0
M-DET.P.m	41
M-DET.P.n	42
S-DET.P.p	17
S-DET.P.r	18
Check D.P.I.	0
Limit value	0.003
Variance	10
Reac.type	Inc

Reaction Rate Method	
Cycle	2
Factor	2
E2 corre	Not do
Blank (u)	9.999
Blank (d)	-9.999
Sample (u)	9.999
Sample (d)	-9.999

Standards Setting	
FV	#
BLK H	9.999
BLK L	-9.999
STD H	9.999
STD L	-9.999