Non-esterified fatty acid (NEFA)

Method: ACS-ACOD

Cat.NO.	Size	Instrument
GB190Z	R1: 1×60ml R2: 1×15ml	Hitachi 7060/7150& ShimadzuCL7200/8000
GS191Z	R1: 1×60ml R2: 1×15ml	Hitachi 7170/7180/7080&AU640
GX191Z	R1:1×60 ml R2:1×15 ml	For Beckman

INTENDED USE

For the quantitative in vitro determination of Non-esterified fatty acids in serum and plasma.

CLINICAL SIGNIFICANCE

Non-esterified fatty acids (NEFA) 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 in blood mainly originate from lipolysis in adipose tissue, which is affected by diet and fluctuations of the insulin level. An increase of the NEFA concentration in pathological states like insulin resistance/diabetes type 2, adiposity, malignant diseases and the metabolic syndrome predict the development of cardiovascular diseases.

ASSAY PRINCIPLE

Non-esterified fatty acids (NEFA) and coenzyme A (CoA) react in the presence of Acyl-CoA 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). At 546nm the intensity of the dye is directly proportional to the concentration of free fatty acids in the sample.

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

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

$2H_2O_2$ +Trinder Substances + 4-AAP $\xrightarrow{POD}$ dye + $4H_2O$

REAGENT COMPOSITON

Contents	Concentration of Solutions
Reagent 1 (R1)	
Phosphate buffer	50 mmol/L
Coenzyme A	0.9mmol/L
ATP	5 mmol/L
Acyl-CoA Synthetase (ACS)	≥0.3kU/L
$MgCl_2$	2 mmol/L
4-AAP	1.5 mmol/L
Reagent 2 (R2)	
Phosphate buffer solution	50 mmol/L
Acyl-CoA Oxidase (ACOD)	≥10 kU/L
Peroxidase (POD)	7.5kU/L

SAMPLE COLLECTION AND PREPARATION

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.

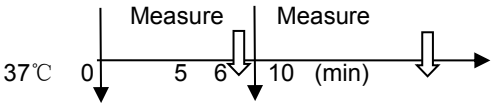
STABILITY AND PREPARATION OF REAGENTS

The reagents and the standard are ready to use.
Stable up to the expiry date when stored at 2-8℃,protected from light and contamination is avoided. Do not freese the reagents.

ASSAY PROCEDURE

Test Procedure for Analyzers (Hitachi7180)
Assay Mode: 2 Point End 15-34
Wave length (sub/main): 600/546nm

Sample 3 µl
R1: 180µl R2: 40 µl



CALIBRATION

We recommend that this assay should be calibrated using the Standard supplied with the kit.

CALCULATION OF RESULTS

NEFA (mg/dl) = ΔA Sample/ΔA Std x Conc. Std (mg/dl)
mg/dl x 0.0354 =mmol/L

QUALITY CONTROL

For quality control, use G-cell NEFA Control as daily quality control sera and can be purchased separately. Values should fall within a specific range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check reaction temperature.
3. Check expiration date of kit and contents.

REFERENCE RANGE

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.

PERFORMANCE CHARACTERISTICS

LINEARITY

The assay is linear up to 3 mmol/L.

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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

Intar assay precision		
N=20	level 1	level 2
Mean(mmol/L)	0.46	1.16
SD	0.01	0.01
CV(%)	1.7	0.69

Inter assay precision			
N=5	Batch 1	Batch 2	Batch 3
Mean(mmol/L)	1.18	1.19	1.20
$\bar{x}$	1.19		
(Xmax-Xmin)/$\bar{x}$	(1.20-1.18)/1.19*100=1.40%		

INTERFERENCE

The following analytes were tested up to levels indicated ang found not to interfere:

Aascorbic acid: up to 50mg/dl
Bilirubin: up to 40mg/dl
Intralipid: up to 300mg/dl
Hemoglobin: up to 300mg/dl

SAFETY PRECAUTIONS AND WARNINGS

1. For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.
2. Reagent contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.
3. Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide from building up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.
4. Specimens should be treated as potentially infectious (HIV, Hepatitis B virus, Hepatitis C virus, etc.) and handled with appropriate caution.
5. Reagents with different lot numbers should not be interchanged or mixed.

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

INDEX OF SYMBOLS

	Manufacture
REF	Catalogue Number
LOT	Lot number
	Date of manufacture
	Use by(Expiration date)
IVD	For In-Vitro Diagnostic use only
2℃ 8℃	Stored at 2-8℃
	Attention:See instruction for use
EC REP	Authorized Representative in the European Company

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