

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<br>1 x 25 ml | Reagent 1<br>Reagent 2 |
| D07950 | 5 x 10 ml | 4 x 10 ml<br>1 x 10 ml | Reagent 1<br>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<br>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) |

\* Advanced Turbidity Clearing System; minimizes turbidity caused by lipemia

### REAGENT COMPOSITION

| COMPONENTS                 | FINAL CONCENTRATION |
|----------------------------|---------------------|
| <b>Reagent 1:</b>          |                     |
| 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 <sub>2</sub>          | 2 mmol/L            |
| Trinder coupling component |                     |
| Detergents and Stabilizers |                     |
| <b>Reagent 2:</b>          |                     |
| 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.<br>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

$$\text{mg/dl} \times 0.0354 = \text{mmol/L}$$

## REFERENCE RANGE [3]

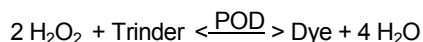
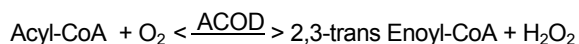
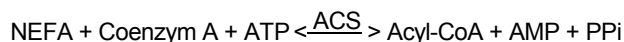
|        |                  |                    |
|--------|------------------|--------------------|
| Women: | 2.8 – 12.7 mg/dl | 0.10 – 0.45 mmol/L |
| Men:   | 2.8 – 16.9 mg/dl | 0.10 – 0.60 mmol/L |

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 coenzyme A synthetase (ACS) to acylated coenzyme A. Acylated coenzyme A is oxidized by acyl coenzyme A oxidase under development of H<sub>2</sub>O<sub>2</sub>. H<sub>2</sub>O<sub>2</sub> is converted to a coloured product by the use of Trinder substances in the presence of peroxidase (POD).



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<br>n = 20 | Mean<br>[mmol/L] | SD<br>[mmol/L] | CV<br>[%] |
|-----------------------|------------------|----------------|-----------|
| 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<br>n = 20 | Mean<br>[mmol/L] | SD<br>[mmol/L] | CV<br>[%] |
|-----------------------|------------------|----------------|-----------|
| 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:



**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:



**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 Endocrinol 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



DIALAB Produktion und Vertrieb von chemisch – technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.  
A – 2351 Wiener Neudorf, Austria  
IZ-NÖ Süd, Hondastrasse, Objekt M55  
Phone: ++43 (0) 2236 660910-0  
Fax: ++43 (0) 2236 660910-30 e-mail: [office@dialab.at](mailto:office@dialab.at)