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For life science research only. Not for use in diagnostic procedures.

Free fatty acids, Half-micro test

Optimized enzymatic colorimetric assay for the determination of free fatty acids (= Non-Esterified Fatty Acids, NEFA) in research samples from serum or plasma.

Cat. No. 11 383 175 001

Test-Combination for approx. 5 × 10 determinations

Version 11

Content version: January 2019

Store at +2 to +8°C

Product overview

Contents

Bottle	Contents
1	• 5 x 11 ml • each of potassium phosphate buffer, pH 7.8
2	• 5 tablets • each tablet contains: ATP, coenzyme A, acyl-CoA-synthetase (Acyl CS), peroxidase, ascorbate oxidase, 4-aminoantipyrine and stabilizers.
3	• 3 ml • ready-to-use! • aqueous N-ethyl-maleinimide solution with stabilizers. ***Note:*** The presence of N-ethyl-maleinimide in the test is necessary for the removal of an existing surplus of CoA before the oxidation of the activated fatty acids by ACOD.
4	• 5 x approx. 0.6 ml • each of ACOD dilution solution and stabilizers.
5	• 5 tablets • each tablet contains: acyl-CoA-oxidase (ACOD) and stabilizers

Test principle

In the presence of the enzyme acyl-CoA synthetase (Acyl CS) and adenosine-5'-triphosphate (ATP), free fatty acids are converted into acyl-coenzyme A (acyl-CoA), adenosine-5'-monophosphate (AMP), and pyrophosphate.

Acyl-CoA reacts with oxygen (0_2) in the presence of acyl-CoA oxidase (ACOD) to form 2,3-enoyl-coenzyme A (enoyl-CoA).

The resulting hydrogen peroxide (H_2O_2) converts 2,4,6-tribromo-3-hydroxy-benzoic acid (TBHB) and 4-aminoantipyrine (4-AA) to a red dye in the presence of peroxidase (POD).

The dye is measured in the visible wavelength range at 546 nm.

$$\text{Free fatty acids} + \text{CoA} + \text{ATP} \xrightarrow{\text{Acyl CS}} \text{acyl-CoA} + \text{AM P} + \text{pyrophosphate}$$

$$\text{Acyl-CoA} + O_2 \xrightarrow{\text{ACOD}} \text{enoyl-CoA} + H_2O_2$$

$$H_2O_2 + \text{4-AA} + \text{TBHB} \xrightarrow{\text{POD}} \text{red dye} + 2\,H_2O + \text{HBr}$$

Application

Determination of free fatty acids (non-esterified fatty acids, NEFA) in serum and plasma in life science research applications.

Interference

Hemoglobin, bilirubin and ascorbic acid do not interfere with the assay if they are present in the normal range.

Storage and stability

The reagents in the unopened bottles are stable at +2 to +8°C until the expiration date printed on the label.

Note: Reaction mixtures A and B are stable for 5 days at +2 to +8°C or for 8 h at +15 to +25°C stored protected from light.

Procedure

Handling instructions

For use with instruments (like Hitachi 704) N-ethyl-maleinimide solution (bottle 3) and Reaction mix B should be mixed at equal volumes. For the assay 0.1 ml of this mixture should be used.

Working solutions

For preparation and stability of working solutions refer to the table below.

Note: Use forceps for taking the tablets out of bottle 2 and 5

.

Solution	Preparation	Stability
Reaction mixture A	Dissolve one tablet of bottle 2 in one bottle 1, sufficient for 10 assays.	Stable for • 5 days at +2 to +8°C • 8 hrs at +15 to +25°C if stored protected from light.
Reaction mixture B	Dissolve one tablet of bottle 5 in one bottle 4, sufficient for 10 assays.	

Sample preparation

- Collect blood from a non-congested vein into a test tube containing EDTA (plasma).
- Prepare serum in the usual way.

Note: Stability of the free fatty acids in serum or plasma: 7 days at +2 to +8°C or 2 days at +15 to +25°C.

Assay conditions

Measurement against air (without a cuvette in the light path) or against water.

Note: If desired, commercially available disposable cuvettes may be used instead of glass cuvettes.

Wavelength	546 nm (Hg)
Half micro glass cuvette	1 cm light path
Temperature	+25°C ± 1°C
Assay volume	1.15 ml

Protocol

Please refer to the following table.

Note: We recommend to mix the reagents in the cuvettes *e.g.* with a plastic spatula or by gentle swirling after closing the cuvettes, *e.g.* with Parafilm .

Step	Action
1	Pipette into cuvettes: **Reagent** / **Blank** / **Sample** Reaction mix A / 1.00 ml / 1.00 ml Sample / - / 0.05 ml Double dist. water / 0.05 ml / - ***Note:*** Rinse the enzyme pipette or pipette tip of the piston pipette with the sample solution before use.
2	• Mix the reagents and bring to +25°C • Keep at this temperature for approx. 10 min.
3	• To each cuvette add 0.05 ml ready-to-use N-ethyl-maleinimide-solution (bottle 3). • Mix and read absorbances of the solutions (A_1).
4	• Start reaction by adding 0.05 ml of Reaction mix B to each cuvette and mix. • Wait for the end of the reaction at +25°C (approx. 15 min) and read absorbances of the solutions (A_2).

Calculation

Calculate the absorbance differences (A_2 -A_1) for both blank and sample. Subtract the absorbance difference of the blank (ΔA_b) from the absorbance difference of the sample (ΔA_s).

This gives ΔA.

$$\Delta A = \Delta A_S - \Delta A_b .$$

Reference values

In serum obtained from up to 3 days old children values between 0.3 and 0.8 mM have been found (2).

Palmitic acid standard solution

If checking the assay by using a standard solution, this may be prepared as follows.

Additional reagents required

- Triton X-100 (Cat. No. 789 704)
- Ethanol, absolute
- Palmitic acid (available from *e.g.* Fa. Sigma, P0500)

Preparation of solutions

Please refer to the following table.

Solution	Preparation/Composition
1	Dissolve 6.0 g of Triton X-100 in about 80 ml of double dist.water (+30° to +40°C), allow to cool to +15 to +25°C and make up to 100 ml in a measuring cylinder.
2	Weigh 9 mg of palmitic acid into a 100 ml beaker and dissolve in about 6 ml of warm ethanol (about +35 to +40°C). Immediately seal the beaker with Parafilm and allow to cool to +15 to +25°C.

Preparation of standard solution

- Add about 80 ml of solution 1 to solution 2, stirring slowly to avoid the formation of microcrystals at the point of entry.
- Stir using a magnetic stirrer for a further 30 min, transfer quantitatively to a 100 ml volumetric flask and make up to the mark with solution 1.

Note: Stable for 3 days when stored at +2 to +8°C (refrigerator). The standard has a concentration of 0.35 mM.

Calculation of the concentration

According to the general formula for calculating the concentration, the equation is:

$$c = \frac{V}{\epsilon \times d \times v} \times \Delta A \text{ [mM sample solution], where:}$$

V =final volume (ml)

v = sample volume (ml)

d =light path (cm)

ε = absorption coefficient of the dye at 546 nm:

$$19.3 \text{ [l} \times \text{mmol}^{-1} \times \text{cm}^{-1}\text{]}$$

Note: The absorbance coefficient of the dye depends on the type of buffer, the pH of the assay system and the purity of TBHB. Under the assay conditions stated above, it varies between 19.1 and 19.5 [l $\times$mmol^{-1} $\times$ cm^{-1}].

It follows for free fatty acids:

$$c = \frac{1.15}{19.3 \times 1 \times 0.05} \times \Delta A = 1.192 \times \Delta A \text{ [mM free fatty acids/serum, plasma]}$$

Note: If checking the assay by using a standard solution, pipette 0.05 ml of standard into the cuvette instead of the sample (serum) as shown in the pipetting scheme.

If deproteinized serum is used in the test, the test volume V in the equation must be corrected by multiplying by the factor F = 0.948.

Dilution factor

The dilution factor is calculated from the specific gravity of the serum or plasma (ρ = 1.03 g/ml) and from the liquid fraction of the serum or plasma (92% = 0.92);

F = 1.03 × 0.92 = 0.948.

Dilution limit

The method shows linearity up to a concentration of 1.5 mM free fatty acids/sample (serum, plasma).

References

1 Shimizu, S. et al. (1980) *Anal. Biochem.* **107,** 193-198.
2 Harris, R. J. (1974)). *Pediatr.* **84,** 578-584.

*available from Roche Diagnostics

Changes to previous version

Editorial changes.

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