iNstruchemie

FFA

FREE FATTY ACIDS - NEFA

DETERMINATION OF FREE FATTY ACIDS (NON ESTERIFIED FATTY ACIDS) IN SERUM OR PLASMA

Enzymatic method
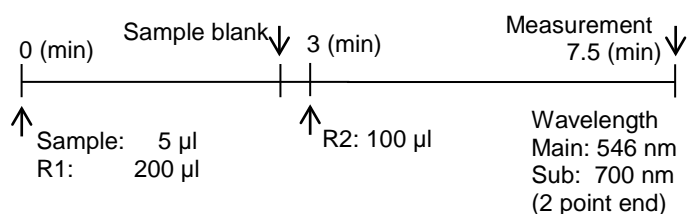
Suitable for all analyzers – 100 tests

Product insert with instructions for automated and manual procedures

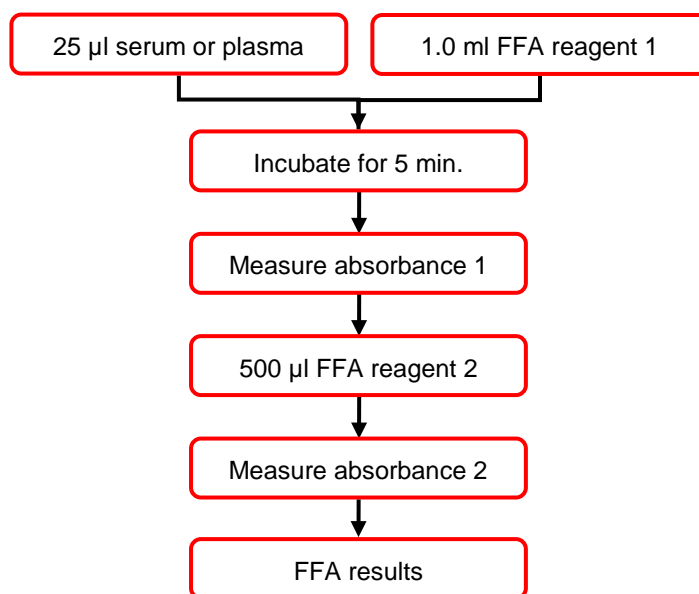Stability > 3 years after production

FFA controls available

Small volumes per vial = Minimized excess reagents

Settings for automatic analyzers

Sample blank ↓

0 (min)   3 (min)   Measurement 7.5 (min) ↓

Sample: 5 µl
R1: 200 µl

R2: 100 µl

Wavelength
Main: 546 nm
Sub: 700 nm
(2 point end)

Added FFA (mmol/l)	Measured (mmol/l)	Recovery (%)
1.11	1.11	100.0
2.22	2.23	100.5
3.33	3.34	100.3

Manual procedure

25 µl serum or plasma → 1.0 ml FFA reagent 1

↓

Incubate for 5 min.

↓

Measure absorbance 1

↓

500 µl FFA reagent 2

↓

Measure absorbance 2

↓

FFA results

Linearity: 4.00 mmol/l   **Mean CV's: 2.04%**   **Mean recovery: 100.3%**

Correlation compared to another manufacturer: 0.997

Product name	Product no.	Quantity
Free Fatty Acids Reagent Set	3055	20 -100 tests
Free Fatty Acids Control High Level	3052	10 x 1 ml
Free Fatty Acids Control Normal Level	3053	10 x 1 ml
Free Fatty Acids Control Extra High Level	3054	10 x 1 ml

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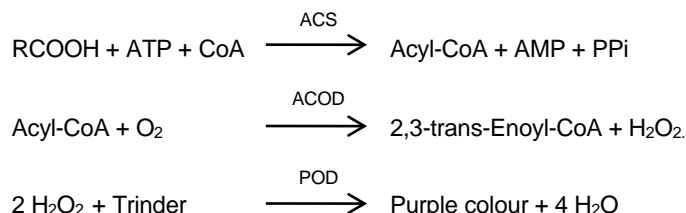
- *Enzymatic method*

- *Suitable for all analyzers – 100 tests*

- *Product insert with instructions for automated and manual procedures*

- *Stability > 3 years after production*

- *FFA controls available*

- *Wavelength 546 nm*

Products	Product no.	Quantity
Free Fatty Acids Reagent Set	3055	20 - 100 tests
Free Fatty Acids Control, High Level	3052	10 x 1 ml
Free Fatty Acids Control, Normal Level	3053	10 x 1 ml
Free Fatty Acids Control, Extra High Level	3054	10 x 1 ml

SUMMARY

PRINCIPLE
Free Fatty Acids react with Coenzyme A in the presence of Acyl CoA Synthetase to Acyl CoA. Acyl CoA is oxidized to 2,3-trans-Enoyl-CoA and H_2O_2. The intensity of the purple pigment is proportional to the concentration of Free Fatty Acids.

$$RCOOH + ATP + CoA \xrightarrow{ACS} Acyl\text{-}CoA + AMP + PPi$$

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

$$2\,H_2O_2. + Trinder \xrightarrow{POD} Purple\ colour + 4\,H_2O$$

SAMPLE MATERIAL
Serum or EDTA Plasma
Stability of the samples: 2 days at 2-6 ºC
Store samples at - 20 ºC when immediate measurement is not possible

LINEARITY
Up to 4.00 mmol/l

EXPECTED VALUES
Serum or EDTA Plasma*:*
Men   : 0.1 – 0.60 mmol/l
Women : 0.1 – 0.45 mmol/l

QUALITY CONTROL
Products	Product no.	Quantity
Free Fatty Acids Control, High Level	3052	10 x 1 ml
Free Fatty Acids Control, Normal Level	3053	10 x 1 ml
Free Fatty Acids Control, Extra High Level	3054	10 x 1 ml

QUANTITY OF DETERMINATIONS
Procedure
- Automated   : 100   tests
- Manual   : 20   tests

NOTES
1.   For in vitro diagnostic use only.
2.   For professional use only.
3.   Contact INstruchemie for the complete design output report and the latest edition product insert.

FFA

CONCENTRATION MEASUREMENTS

The concentrations of a normal high and extra high sample were measured with an automatic analyzer in order to verify acceptable absorbances.
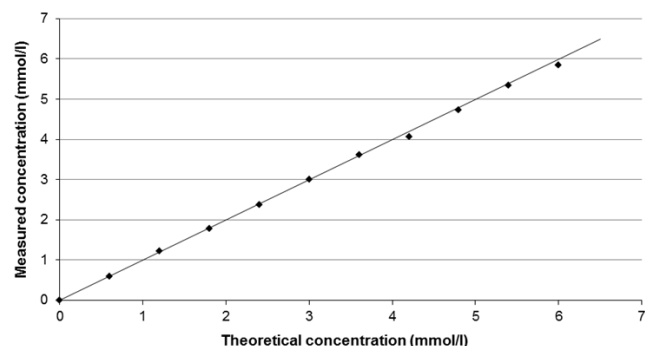
FFA measurements

	Low	Normal	High
Absorbance	0.0258	0.109	0.216
Concentration (mmol/l)	0.19	0.80	1.57

TEST CONDITIONS

All tests were performed under the following conditions:

Temperature	: 37 °C
Wavelength	: 546 nm
Light path	: 0.7 cm
Blank	: Distilled or deionized water
Sample	: Serum / EDTA plasma

SENSITIVITY

The sensitivity (limit of detection) was determined by measuring basic calibrator material
(FFA concentration = 0 mmol/l) 20 times.

Sensitivity = 3 x standard deviation = 3 x 0.003 = 0.009 mmol/l

LINEARITY

The FFA assay is at least linear up to 4.00 mmol/l.

FFA linearity measurements with an automatic analyzer

RECOVERY

The recovery is determined by measuring the FFA concentration in a spiked human serum sample 10 times using an automatic analyzer.

Recovery:

Added FFA (mmol/l)	Measured (mmol/l)	Recovery (%)
1.11	1.11	100.0
2.22	2.23	100.5
3.33	3.34	100.3

PRECISION

The precision is determined by measuring a serum sample and Control Extra High Level 10 times a day (repeatability) for 5 consecutive days (reproducibility), using an automatic analyzer.

Repeatability:

	Sample (mmol/l)	Control (mmol/l)
Mean	0.24	1.51
Standard deviation	0.006	0.027
Variation coefficient (%)	2.50	1.79

Reproducibility:

	Sample (mmol/l)	Control (mmol/l)
Mean	0.24	1.50
Standard deviation	0.005	0.027
Variation coefficient (%)	2.08	1.80

CORRELATION

Pearsons' correlation is determined by measuring the FFA concentration in multiple human sera with INstruchemie reagent and reagent of another manufacturer.

FFA correlation measured with an automatic analyzer (mmol/l)

n = 10

r = 0.997

y = 0.9362x + 0.0126
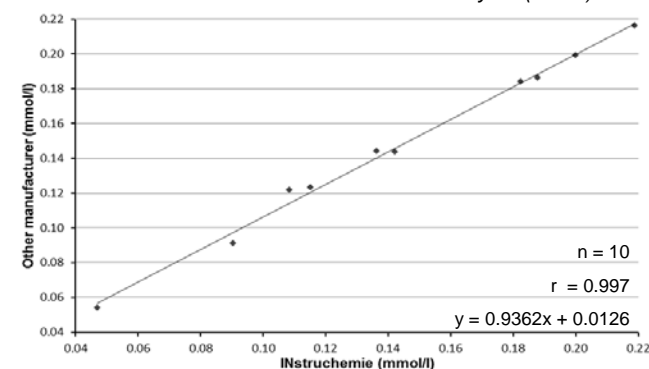
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