NEFA

Non-Esterified Fatty Acids
MANUAL
RX MONZA

INTENDED USE

For the quantitative *in vitro* determination of Non-Esterified Fatty Acids (NEFA) in serum and plasma. This product is suitable for manual use and on the RX **monza** analyser.

Cat. No.			
FA 115	R1a.	Buffer	1 x 70 ml
3 x 10 ml	R1b.	Enzyme/Coenzymes	3 x 10 ml
	R2a.	Enzyme Diluent	3 x 20 ml
	R2b.	Maleimide	3 x 20 ml
	R2c.	Enzyme Reagent	3 x 20 ml
	CAL.	Standard	1 x 5.5 ml

GTIN: 05055273203066

COLORIMETRIC METHOD

PRINCIPLE (3,5,6)

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

$$Acyl\ CoA + 0_2 \xrightarrow{\text{Acyl CoA Oxidase}} 2,3,\text{-trans-Enoyl-CoA} + H_20_2$$

$$2H_20_2 + TOOS + 4\text{-}AAP \xrightarrow{\text{Peroxidase}} \text{purple adduct} + 4H_20$$

4-AAP = 4-aminoantipyrine
TOOS = N-ethyl-N-(2hydroxy-3-sulphopropyl) m-toluidine

SAMPLE (1)

Serum, plasma.
Do not use heparinized plasma as heparin has been found to interfere with the assay. Suitable anti-coagulants are as follows:
EDTA
Sodium citrate
Sodium fluoride
Ammonium oxalate

REAGENT COMPOSITION

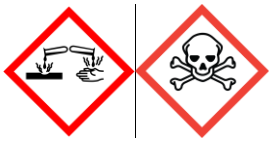
Contents		Initial Concentration of Solutions
R1a.	**Buffer**	
	Phosphate Buffer	0.04 mol/l, pH 6.9
	Magnesium Chloride	3 mmol/l
	Surfactant	
R1b.	**Enzyme/Coenzymes**	
	Acyl Coenzyme A Synthetase	≥0.3 U/ml
	Ascorbate oxidase	≥1.5 U/ml
	Coenzyme A	0.9 mmol/l
	ATP	5.0 mmol/l
	4-aminoantipyrine	1.5 mmol/l
R2a.	**Enzyme Diluent**	
	Phenoxyethanol	0.3 % (w/v)
	Surfactant	
R2b.	**Maleimide**	10.6 mmol/l
R2c.	**Enzyme Reagent**	
	Acyl Coenzyme A Oxidase	≥10 U/ml
	Peroxidase	7.5 U/ml
	TOOS	1.2 mmol/l
CAL.	**Standard**	See lot specific insert.

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

This kit contains components classified as hazardous in accordance with the Regulation (EC) No.1272/2008:

R2b. Maleimide



DANGER
Toxic if swallowed.
Causes severe skin burns and eye damage. May cause an allergic skin reaction.

Precautions:
Avoid breathing the mist/vapours/spray.
Wear protective gloves/ protective clothing/ eye protection/face protection.
Wash thoroughly after handling.

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Immediately call a POISON CENTER/doctor.

Collect spillage. Avoid release to the environment. Disposal of all waste material should be in accordance with local guidelines.

CAL. Standard

DANGER

Flammable liquid and vapour.
Fatal if swallowed. Causes serious eye damage.
Harmful to aquatic life with long lasting effects.

Precautions:
Keep away from heat.

IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
If swallowed call a doctor.

Avoid release to the environment. Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available on request.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

R1a. Buffer
Contents ready for use. Stable up to the expiry date when stored at +2 to +8°C.

R1b. Enzyme/Coenzymes
Reconstitute the contents of one vial of Enzyme/ Coenzymes R1b with **10 ml** of Buffer R1a. Stable for 5 days at +2 to +8°C. Do not freeze. Protect from light.

R2a. Enzyme Diluent
Contents supplied ready for use. Stable up to the expiry date when stored at +2 to +8°C.

R2b. Maleimide
Reconstitute the contents of one bottle of Maleimide R2b with the entire contents of one bottle of Enzyme diluent R2a. Ensure that Maleimide is completely dissolved. Use immediately to reconstitute bottle R2c.

R2c. Enzyme Reagent
Reconstitute the contents of one vial of Enzyme Reagent R2c with one bottle of Solution R2b. Stable for 5 days at +2 to +8°C. Do not freeze. Protect from light.

CAL. Standard
Contents supplied ready for use. Stable up to the expiry date when stored at +2 to +8°C.

R1= Buffer/ Enzyme/ Coenzymes
R2 = Enzyme Diluent/ Maleimide/ Enzyme Reagent

MATERIALS PROVIDED

Buffer
Enzyme/Coenzymes
Enzyme Diluent
Maleimide
Enzyme Reagent
Standard

MATERIALS REQUIRED BUT NOT PROVIDED

Randox Assayed Multi-sera Level 2 (Cat. No. HN 1530) and Level 3 (Cat. No. HE 1532)

NOTES (1-7)

1. Visibly lipaemic specimens require a sample blank.
2. Samples with bilirubin or haemoglobin above the following levels, require a sample blank:

	Maximum Level Allowed for Test Accuracy		Effect on Result
Bilirubin	10	mg/dl	Decreased
Haemoglobin	100	mg/dl	Increased

These samples will require a sample blank* to be performed ($A_{sample\ blank}$). For Manual Procedure the NEFA concentration will then be calculated as follows: -

$$mmol/l = \frac{A_{sample} - A_{sample\ blank}}{A_{standard}} \times \text{std. Conc.}$$

For RX **monza** the NEFA concentration will be calculated as follows:

Sample - Sample Blank = Sample Concentration (mmol/l)

3. Over-recovery of Quality Control Material may be observed when reading monochromatically. To correct this take additional readings for A_{sample} and $A_{standard}$ at 700nm, and subtract from the main wavelength readings.
4. The sample to be tested must not be heparinized, as this stimulates lipoprotein lipase activity, thereby causing the release of NEFA from triglycerides associated with blood lipoproteins. Consequently, blood collected from patients receiving therapeutic heparin or blood collected in heparin containers, is not suitable for this test.
5. Due to the inclusion of ascorbate oxidase in Enzyme Reagent A, ascorbic acid at levels > 20 mg/dl (i.e. > 10 times the normal value) do not interfere with the test.
6. NEFA determinations must be carried out on serum derived from fasting individuals, otherwise the result cannot be directly compared to a normal range of fasting controls.
7. If a serum sample remains at room temperature for a significant length of time, the level of NEFA within it, will increase due to enzymatic action. Therefore, failing immediate analysis, serum samples can be frozen at -20°C for a maximum of 24 hours.
8. If $A_{standard} - A_{reagent\ blank} < 0.180$ repeat the assay with fresh reagent.

RX MONZA PROCEDURE

Select NEFA in the Run Test screen and carry out a water blank as instructed.

Pipette into a test tube:				
	Reagent Blank S0	Standard S1	Sample	*Sample Blank
dd H_2O	10 µl	-	-	-
Standard	-	10 µl	-	-
Sample	-	-	10 µl	-
Reagent 1	200 µl	200 µl	200 µl	200 µl
Mix, incubate for 5 minutes at +37°C.				
Reagent 2	400 µl	400 µl	400 µl	400 µl
Sample	-	-	-	10 µl

Mix, incubate for 5 minutes at +37°C.
Insert the cuvette into the RX **monza** flowcell holder and press Read.
* See Notes 1 and 2.

MANUAL PROCEDURE (6)

Wavelength:	550 nm
Cuvette:	1 cm light path
Temperature:	+37°C
Measurement:	against reagent blank

Pipette into cuvette:

	Reagent Blank	Standard	Sample	*Sample Blank
Distilled Water	50 µl	-	-	-
Standard	-	50 µl	-	-
Sample	-	-	50 µl	-
Solution R1	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Mix, and incubate at +37°C for 10 minutes.				
Solution R2	2.0 ml	2.0 ml	2.0 ml	2.0 ml
Sample	-	-	-	50 µl

Mix, and incubate at +37°C for 10 minutes. Read absorbance of sample (A_{sample}) and standard ($A_{standard}$) against the reagent blank at 550 nm.

* See Notes 1, 2 and 3.

N.B. Read time must be exactly 10 mins.

CALCULATION

1. Using a Calibration Curve

The calibration curve should be confirmed for each new lot of reagents by setting up the following:

Tube No	1	2	3	4
Name	Blank	Low Std	Normal Std	High Std
NEFA Std	-	25 µl	50 µl	100 µl
Water	50 µl	25 µl	-	-
Solution R1	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Mix, and incubate at +37°C for 10 minutes.				
Solution R2	2.0 ml	2.0 ml	2.0 ml	2.0 ml
Mix, and incubate at +37°C for 10 minutes.				
Abs	0.000	(read)	(read)	(read)
*Std Value	0.000	Std value x 0.250	Std value x 0.500	Std value x 1.000

*Standard value can be found in the lot specific insert included in the kit.

Plot absorbance ($A_{550\ nm}$) verses NEFA concentration (mmol/l). This should be a straight line as this follows Beer's Law and is linear over the range from 0.0 - 2.0 mmol/l.

2. Using a Standard

The NEFA concentration in a sample may be determined using the following equation.

$$\text{mmol/l} = \frac{A_{sample}}{A_{standard}} \times \text{Concentration of standard}$$

QUALITY CONTROL

Randox Assayed Multi-sera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified 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 cleanliness of all equipment in use.
3. Check water. Contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Customer Technical Services, Northern Ireland +44 (0) 28 94451070.

NORMAL RANGE (2,8)

Fasting: 0.1 - 0.9 mmol/l

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

INTERFERENCE

Nefa and Triglycerides should not be tested in the same run.

Physiological changes in serum or plasma analyte concentrations can be caused by a number of substances. Comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. The listed reference contains specific details on known potential interfering substances(9). The user must remain vigilant to the possible effect on results of unknown interferences from medications or endogenous substances. All patient results must be evaluated in light of the total clinical status of the patient.

SPECIFIC PERFORMANCE CHARACTERISTICS

The following NEFA performance characteristics were obtained using the RX **monza** analyser in cuvette mode running at a temperature of +37°C.

LINEARITY

The method is linear up to 2.24 mmol/l. Samples with higher concentrations should be diluted 1 + 3 with 0.9% NaCl solution and reassayed. Multiply the result by 4.

SENSITIVITY

The minimum detectable level with acceptable precision has been determined as 0.072 mmol/l.

PRECISION

Within Run Assay

	Level 1	Level 2
Mean (mmol/l)	1.73	1.20
SD	0.082	0.058
CV (%)	4.74	4.81
n	20	20

Total Assay

	Level 1	Level 2
Mean (mmol/l)	1.73	1.20
SD	0.078	0.052
CV (%)	4.51	4.32
n	20	20

CORRELATION

This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:

Y = 1.0372 X + 0.0457
and a correlation coefficient of r = 0.9855

49 patient samples were analysed spanning the range 0.09 to 2.08 mmol/l.

REFERENCES

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The presence of a vertical bar in the margin indicates a technical update from the previous revision.

EC REP

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