CATACHEM

NONESTERIFIED FATTY ACIDS (NEFA) REAGENT KITS
C514-0A, V514-0B
FOR VETERINARY USE ONLY

Contents	Product No.	Package
NEFA REAGENT KIT	**C514-0A**	
Sample Diluent (R1)	C514-01	3 x 30 mL
Enzyme Color Reagent (R2)	C514-02	3 x 15 mL
Reagent Diluent	C514-03	1 x 145 mL
NEFA REAGENT KIT	**V514-0B**	
Enzyme Sample Reagent (R1)	V514-01	3 x 8 mL
Enzyme Color Reagent (R2)	V514-02	3 x 4 mL
Reagent Diluent	V514-03	2 x 25 mL

REAGENT PREPARATION (C514-0A)

Reconstitute one vial of Sample Diluent R1 (C514-01) with 30 mL of Reagent Diluent (C514-03). Mix gently to prevent foaming until all material is in the solution. USE WITHIN 24 HOURS.

Reconstitute one vial of Enzyme Color Reagent R2 (C514-02) with 15 mL of Reagent Diluent (C514-03). Mix gently to prevent foaming until all material is in solution. Reconstituted material is stable for 30 days at 2-8°C.

REAGENT PREPARATION (V514-0B)

Reconstitute one vial of Enzyme Sample Reagent R1 (V514-01) with 8 mL of Reagent Diluent (V514-03). Mix gently to prevent foaming until all material is in solution. Use within 24 hours.

Reconstitute one vial of Enzyme Color Reagent R2 (V514-02) with 4 mL of Reagent Diluent (V514-03). Mix gently to prevent foaming until all material is in solution.

Store both reconstituted reagents at 2-8°C.

REAGENT STORAGE AND STABILITY

Store unopened and un-reconstituted reagents at 2-8°C.
When stored as directed, reagents are stable until the expiration date stated on the label.

NOT FOR USE IN UNPROFESSIONAL SETTINGS

FOR TECHNICAL ASSISTANCE:
Email: catachem@catacheminc.com
Contact Form: www.catacheminc.com
Call: +1 203-262-0330

NONESTERIFIED FATTY ACIDS (NEFA) REAGENT KITS
C514-0A, V514-0B
FOR VETERINARY USE ONLY
MANUAL/AUTOMATED APPLICATION

Intended Use
For **In Vitro Diagnostic** use in the manual or automated, quantitative, determination of Non esterified Fatty Acids (NEFA) in serum or plasma.

Clinical Significance ([1])
Fatty acids are absorbed through the intestine as insoluble oils. Solubilization (emulsification) is accomplished by the presence of bile salts synthesized in the liver. In the blood, this important lipid form is carried by albumin. Problems with fatty acid metabolism are associated with the process of fatty acid ß-oxidation, carnitine deficiency which is observed in newborns, and patients undergoing dialysis. Deficiency of the enzyme Carnitine Palmitoyl transferase results in recurrent muscle pain, fatigue and myoglobinuria. Another enzyme deficiency, acyl–CoA Dehydrogenase, will cause symptoms of vomiting, lethargy and frequently coma after a prolonged fasting period. In Refsum disease, accumulation of Phytanic Acid due to blockage of ß-oxidation results in cerebellar ataxia, retinitis pigmentosa, nerve deafness and peripheral neuropathy.

Method Principle ([2-4])
Most previously used procedures for Non esterified Fatty Acids were based on organic solvent extractions, titration and gas liquid chromatography. These procedures are complicated, time consuming and restricted to manual assays. Catachem's NEFA procedure is based on the enzymatic synthesis of thiol esters of CoA (Co-enzyme A), known as Acyl-CoA, by the activity of Acyl-CoA Synthetase (ACS) in the presence of ATP and CoA. The Acyl-CoA thus formed is then oxidized in a second reaction by Acyl-CoA Oxidase (ACOX) to produce 2, 3-trans-trans-enoyl-CoA and Hydrogen Peroxide. The Hydrogen Peroxide is then quantified by the oxidative condensation of N-Ethyl-(3-sulfopropyl) Aniline (ALPS) with 4-Aminoantipyrine to produce a Quinone indamine dye with maximum absorption at 550nm. The increase in absorbance is directly proportional to the concentration of NEFA in the original serum sample. The following reaction scheme illustrates the reactions that take place in this NEFA procedure.

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

$$\text{Acyl-CoA} + \text{O}_2 + \xrightarrow{\text{ACOX}} \text{2,3-trans-Enoyl-CoA} + \text{H}_2\text{O}_2$$

$$\text{H}_2\text{O}_2 + 4\text{AA} + \text{ALPS} \xrightarrow{\text{Peroxidase}} \text{Quinoneindamine Dye} + 4\text{H}_2\text{O}$$

SAMPLE DILUENT (R1)
Each liter contains:

Buffer	
ATP	4.0 mmol
4-Aminoantipyrine	1.0 mmol
Coenzyme-A (CoA)	350 mg
Ascorbate Oxidase	≥ 3000 U
Acyl-CoA Synthetase	≥ 150 U

ENZYME COLOR REAGENT (R2)
Each liter contains:

Buffer	
ALPS	1.62 mmol
Acyl-CoA Oxidase	≥ 15000 U
Peroxidase	≥ 15000 U
Stabilizer and nonreactive ingredients	

REAGENT DILUENT
Each liter contains:

Magnesium Chloride	3.0 mmol
Surfactant	
Stabilizer and nonreactive ingredients	

Precautions
Handle this reagent using good laboratory practice. **DO NOT PIPETTE REAGENT BY MOUTH.** Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Clean spills immediately.

Reagent Storage and Stability
Store Catachem's NEFA reagents at 2-8°C. When stored as directed, these reagents are stable until the expiration date stated on the label. The Catachem NEFA Reagent has been tested to reflect shipping conditions and is stable for the lifespan of the product if frozen up to 5 times or upon reaching temperatures of up to 40°C for up to one week.

Working Reagent Preparation
R1 and R2 Working reagents are prepared as previously noted. Store the Working Reagents at 2-8°C. When prepared as directed, the NEFA R1 Working Reagent should be used within 24 hours whereas the NEFA R2 reagent is stable for 30 days.

Reagent Indications of Deterioration
- Turbidity
- Absorbance > 0.5 OD, 1 cm light path - 550 nm
- Quality control values out of assigned ranges

If these reagent characteristics are observed, contact Catachem's technical service.

Specimen Collection and Stability
Test sera should be collected after fasting and should be fresh, clear and non-hemolyzed. When blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay. If immediate assays are not possible samples should be frozen at -20°C. If plasma specimens are collected, do not use heparin as it is known to interfere with the NEFA reaction. Anticoagulants suitable for this assay are sodium citrate, EDTA, and sodium fluoride.

Procedure
These instructions are outlined for performing Catachem's NEFA assay using a manual procedure. Contact Catachem's Technical Service if instrument applications are required.

Materials Provided
Catachem's NEFA Reagents

Materials required but not provided
- Spectrophotometric analyzer equipped with 550 nm wavelength
- NEFA Calibrator material with assigned value
- NEFA Quality Control material with assigned values

Calibration
Catachem's NEFA Calibrator (C514-10) which contains a known NEFA value is recommended.

Assay Procedure
1. Label cuvettes or appropriate test tubes as: a) Calibrator Blank (CAL-BLK), b) Calibrator (CAL), c) Control 1 Blank (C-1BLK),

353 Christian Street, Suite 2 ● Oxford, Connecticut ● 06478 USA ● Phone (203) 262-0330 ● catachem@catacheminc.com

d) Control 1 (C1), e) Control 2 Blank (C-2BLK), f) Control 2 (C2), g) Sample Blank (SAMP BLK), h) Sample (SAMP).
2. Pipette the reagent and sample volumes into the cuvettes or test tubes as shown in table below. First pipette Catachem's NEFA Enzyme Reagent I (R-1), followed by the sample.
3. Incubate for 5 minutes.
4. Immediately, after the incubation period is over, add Catachem's NEFA Enzyme Reagent II (R-2) to the assay samples and water to the blank samples. Mix all cuvettes.
5. Set a timer for exactly 4 minutes.
6. At the end of the 4 minutes, read all cuvettes at 550 nm. Record all absorbencies.

	CAL BLK	CAL	C-1 BLK	C-1	C-2 BLK	C-2	SAMP BLK	SAMP
	ml	ml	ml	ml	ml	ml	ml	ml
RGT 1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
SAMP	0.050	0.050	0.050	0.05	0.050	0.05	0.050	0.050
INCUBATE FOR 5 MINUTES								
RG2 2	0.00	0.25	0.00	0.25	0.00	0.25	0.00	0.25
H2O	0.10	0.0	0.10	0.00	0.10	0.00	0.10	0.00
MIX AND INCUBATE FOR 4 MINUTES								
MIX AND READ ALL CUVETTES								

Calculations and Results

$$\text{NEFA mmol/L} = \frac{\Delta\text{- Abs. Samp.}}{\Delta\text{- Abs. Cal.}} \times \text{Cal. Value (mmol/L)}$$

Example:

	Samp. Abs.	Blk. Abs.	Δ-Abs.
Sample	0.400	0.300	0.100
Calibrator	0.150	0.030	0.120

Calibrator assigned value = 0.5 mmol/L

$$\text{Sample NEFA mmol/L} = \frac{0.100}{0.120} \times 0.5 \text{ mmol/L}$$

$$= 0.42 \text{ mmol/L}$$

Quality Control

To ensure optimal performance of these reagents and this procedure, we recommend systematic calibration using Catachem's NEFA Calibrator (C514-10). Assay performance should be monitored by running normal/abnormal controls concomitantly with samples. To monitor the quality performance of the procedure followed, Catachem's NEFA Control Level I and Control Level II should be included in the assay procedure each time the assay is run.

Procedure Limitations

Samples with NEFA values greater than 2.5 mmol/L should be diluted 1:2 with physiological saline and re-assayed. Multiply results obtained by 2 to adjust for the sample dilution.

Interfering Substances

The following substances have no significant effect on the accuracy of this NEFA procedure at the concentrations stated.

- Hemoglobin ≤ 200 mg/dL
- Bilirubin ≤ 10.0 mg/dL
- Ascorbic Acid ≤ 20 mg/dL

Other substances and certain drugs are also known to influence the NEFA values [6].

Method Performance Characteristics

Sensitivity: Using a path length of 1 cm, a Δ-absorbance of 0.1-0.20 per mmol/L should be obtained.
Linearity: This procedure is linear over the range of 0-2.5 mmol/L.
Precision: Precision data was obtained using five levels of protein-based controls and following the NCCLS EP5-T2 procedure (5). The following results were observed:

Precision

NEFA	Within-Run Precision		Total Precision	
Mean	SD	CV	SD	CV
mmol/L	mmol/L	%	mmol/L	%
0.169	0.0060	3.550	0.0074	4.379
0.443	0.0087	1.953	0.0100	2.257
1.02	0.0149	1.461	0.0162	1.588
1.516	0.0163	1.078	0.0172	1.134

ACCURACY

Using an automated analyzer, correlation studies were carried out between this Catachem's NEFA procedure (Y) and a commercially available NEFA test kit as reference (^{X}). Serum samples were assayed and the results compared by the least squares regression. The following statistics were observed:

N	=	44
Range	=	0.1-2.3 mmol/L
Mean Y	=	0.5295 mmol/L
Mean X	=	0.5841 mmol/L
Y	=	0.9137x – 0.0042
r	=	0.9949
Sy.x	=	0.0541

Bibliography

1. Fundamentals of Clinical Chemistry. Edited by Norbert Tietz 2nd. Ed. Philadelphia: W.B Saunders; 1976.
2. Itaya, K and Ui, M. Colorimetric determination of free fatty acids in biological fluids. J. Lipids Research; 6, 16 (1965).
3. Novak, M. Colorimetric ultramicro method for the determination of fatty acids. J. Lipid Research; 6, 431 (1965).
4. Trout, D.L., Estes, E.H. and Friedberg, S.J. J. Lipid Research; 1,199 (1960).
5. Evaluation of Precision Performance of Clinical Chemistry Devices. Second Edition. NCCLS Document EP5-T2. Vol. 12, No. 4.
6. Young D.S., Pestamer L.C., Gibberman V. Effects of drugs on clinical laboratory tests. Clin. Chem. 21, No. 5(1975).

353 Christian Street, Suite 2 ● Oxford, Connecticut ● 06478 USA ● Phone (203) 262-0330 ● catachem@catacheminc.com