Instructions for Use
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Non-esterified Free Fatty Acids (NEFA) Assay Kit

Catalog No.: abx294013

Size: 100 tests

Detection range: 0.05 mmol/L – 2.0 mmol/L

Storage: Store all kit components at 2-8°C.

Application: For detection and quantification of non-esterified free fatty acids (NEFA) concentration in serum and tissue homogenates.

Introduction

Non-esterified fatty acids, also known as free fatty acids, are derived from metabolism of adipose tissue and the diet. In adipose tissue, triglycerides are decomposed by hormone-sensitive lipase (HSL) to produce fatty acids and glycerol. Free fatty acids that circulate in the body become combined with plasma albumin and are used as an energy source utilized by tissues and organs, such as the brain and muscles. Non-esterified fatty acids are also the substrate of fat synthesis; therefore, the concentration of non-esterified fatty acids is related to endocrine function, lipid metabolism and glucose metabolism. In a weak acidic condition, non-esterified fatty acid will react with nantokite to form copper soap.

Abbexa's Non-esterified Free Fatty Acids (NEFA) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Non-esterified Free Fatty Acids concentration. The product has an absorbance maxima at 715 nm. The intensity of the color is proportional to the Non-esterified Free Fatty Acids concentration, which can then be calculated.

Kit components

1. Extraction Solution: 3 × 60 ml
2. Palmitic Acid Standard (10 mmol/L): 2 × 1.8 ml
3. Control Solution: 28 ml
4. Reaction Solution: 45 ml

Materials Required But Not Provided

1. Spectrophotometer and cuvettes
2. PBS (0.01 M, pH 7.4)
3. Pipette and pipette tips
4. Vials/tubes
5. Centrifuge
6. Vortex mixer
7. Incubator
8. Ice
9. Fume cupboard

For Reference Only

Protocol

A. Preparation of samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position at 25°C for 30 minutes. Centrifuge at approximately 2000 × g for 15 mins at 4°C. Take 0.2 ml of the serum and add 2.4 ml of Extraction Solution (in a 1:12 ratio). Mix with an orbital shaker at 4°C for 3 hours to extract the NEFA. Centrifuge the sample at 2000 × g for 10 minutes at 4°C. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh 200 mg of tissue and wash with pre-chilled PBS. Remove the water on the surface of the tissue with absorbent paper. Homogenize 0.2 g of tissue in 2.4 ml of Extraction Solution at 4°C (in a 1:12 ratio). Mix with an orbital shaker at 4°C for 2 hours to extract the NEFA. Centrifuge the homogenate at 10000 × g at 4°C for 10 min. Collect the supernatant, keep on ice and assay immediately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Rat Liver Tissue Homogenate	1-3
Rat Heart Tissue Homogenate	1
Rat Kidney Tissue Homogenate	1
Mouse Liver Tissue Homogenate	1-3
Rat Serum	1
Mouse Serum	1
Human Serum	1

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Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for tissue homogenates.
- The diluent is Extraction Solution.

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B. Assay Procedure

1. Set control, standard, and sample tubes label accordingly. It is recommended to use 2 control and 2 standard tubes for each assay run.
2. Dilute the 10 mmol/L standard solution with the Extraction Solution to create a standard curve. The recommended dilution gradient is as follows: 0, 0.05, 0.1, 0.25, 0.5, 1.0, 1.5, and 2.0 mmol/L. Please see the below table as a reference.

Standard Concentration (mmol/L)	10 mmol/L Standard Solution (µl)	Extraction Solution (µl)
0	0	3000
0.05	15	2985
0.1	30	2970
0.25	75	2925
0.5	150	2850
1.0	300	2700
1.5	450	2550
2.0	600	2400

3. Add 1 ml of each standard to the corresponding standard tubes.
4. Add 1 ml of sample to the control tubes.
5. Add 1 ml of sample to the sample tubes.
6. Add 0.5 ml of Control Solution to each control tube.
7. In a fume cupboard, add 0.5 ml of Reaction Solution to each standard tube and each sample tube.
8. Mix by vortexing for 5 minutes and stand at room temperature for 5 minutes.
9. Zero the spectrophotometer using the Extraction Solution at 715 nm.
10. Add 0.8 ml of supernatant of each tube into a 1 ml cuvette and measure the OD value at 715 nm.

C. Calculation of Results

The standard curve can be plotted as the absolute OD_{450} of each standard solution (y) vs. the respective concentration of the standard solution (x). A linear fit is recommended for the standard curve (y = ax + b). Create the standard curve with graph software.

1. Serum samples:

$$\text{NEFA (mmol/L)} = (\Delta A_{715} - b) \times \frac{V_1}{a \times V_2} \times f$$

2. Tissues samples:

$$\text{NEFA (µmol/g)} = (\Delta A_{715} - b) \times \frac{V_3}{a \times m} \times f$$

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where:

y	$\mathrm{OD}_{\mathrm{Standard}} - \mathrm{OD}_{\mathrm{Blank}}$
x	The concentration of the standard
a	The slope of the standard curve
b	The intercept of the standard curve
$\Delta\mathrm{A}_{715}$	$\mathrm{OD}_{\mathrm{Sample}} - \mathrm{OD}_{\mathrm{Control}}$
V_1	The volume of the Extraction Solution added during the pretreatment of the serum samples (2.4 ml)
V_2	The volume of serum (0.2 ml)
V_3	The volume of the Extraction Solution added during the pretreatment of the tissue samples (2.4 ml)
m	The weight of the tissue (0.2 g)
f	Dilution factor of sample before carrying out the assay

For Reference Only