Non-Esterified Free Fatty Acid Assay Kit

Catalog No.: abx298939

Size: 96 tests

Detection range: 0.15 µmol/ml – 1.5 µmol/ml

Sensitivity: 0.15 µmol/ml

Storage: Store all components at 4°C.

Application: For detection and quantification of NEFA concentration in tissue homogenate and cell lysate.

Introduction

Non-Esterified Free Fatty Acids (NEFAs) are components of triglyceride fats that are freed by the action of the enzyme lipase in adipose tissues. Structurally, NEFAs contain an aliphatic carbon chain, which can be saturated (contains no carbon-carbon double bonds) or unsaturated (contains a carbon-carbon double bond), and end with a carboxylic acid group. NEFAs are circulated through the body in the blood, bound to the protein albumin. These complexes can be used as a metabolic fuel source by cells, particularly in heavily respiring organs like the brain and the muscles. Changes in the concentration of NEFAs can be used as an indicator of the rates of lipid and glucose metabolism in a biological system.

Abbexa's Non-Esterified Free Fatty Acid Assay Kit is a quick, convenient, and sensitive method for measuring and calculating NEFA concentration. Under acidic conditions, NEFAs will react with the cupreous mineral nantokite to form a copper soap, which absorbs light at 715 nm. The amount of absorbance of this compound at 715 nm is proportional to the concentration of NEFA in the sample. The concentration of NEFA can be calculated by measuring the absorbance at 715 nm.

Kit components

1. 96-well microplate
2. Standard (10 µmol/ml Palmitic Acid): 2 × 1 ml
3. Control Solution: 12 ml
4. Reaction Solution: 20 ml
5. Extraction Solution: 2 × 60 ml
6. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (715 nm)
2. Pipette and pipette tips
3. Centrifuge
4. Microcentrifuge tubes
5. Incubator
6. Homogenizer, glass homogenizing tube, or mortar and pestle
7. Microtube vortex mixer or oscillator

Protocol

A. Preparation of Samples and Reagents

1. Samples

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

- **Fresh Tissue:** Wash the tissue with PBS kept on ice (4°C, 0.01 M, pH 7.4), then pat dry with absorbent towels. Ensure the sample does not dry out fully. Weigh, then mince the tissue. Add Extraction Solution in a 1:12 ratio of sample to buffer (i.e. for every 1 g of tissue, add 12 ml of Extraction Solution). Homogenize the mixture. This can be done manually in a homogenizing tube by grinding the minced tissue with a glass rod for 6-8 minutes in the tube on ice, or in a mortar and pestle with liquid nitrogen. Alternatively, this step can be performed mechanically using a homogenizer at 60 Hz for 90 seconds in an ice bath. Vortex the resulting solution at 4°C for 2 hours, then centrifuge at 10,000 × g for 10 minutes at 4°C. Carefully remove the supernatant and transfer it to a fresh tube. Keep on ice and analyze immediately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Extraction Solution, then carry out the assay procedure. The concentrations of each sample must fall within the kit's stated range (0.15 µmol/ml – 1.5 µmol/ml).

A dilution factor of 1 is recommended Rat liver, heart, kidney, and Mouse liver tissue homogenate.

All samples should be collected fresh and analyzed within 24 hours. Keep samples at 4°C and avoid freezing.

2. Reagents

- **Palmitic Acid Standard Solutions:** Label 8 tubes with 1.5 µmol/ml, 1.2 µmol/ml, 1.0 µmol/ml, 0.9 µmol/ml, 0.6 µmol/ml, 0.4 µmol/ml, 0.3 µmol/ml, and 0 µmol/ml. Add 225 µl, 180 µl, 150 µl, 135 µl, 90 µl, 60 µl, and 45 µl of Standard (10 µmol/ml) to the 1.5 µmol/ml, 1.2 µmol/ml, 1.0 µmol/ml, 0.9 µmol/ml, 0.6 µmol/ml, 0.4 µmol/ml, and 0.3 µmol/ml tubes respectively, followed by 1275 µl, 1320 µl, 1350 µl, 1365 µl, 1410 µl, 1440 µl, and 1455 µl of Extraction Solution, to prepare the Standard Solutions with concentrations of 1.5 µmol/ml, 1.2 µmol/ml, 1.0 µmol/ml, 0.9 µmol/ml, 0.6 µmol/ml, 0.4 µmol/ml, and 0.3 µmol/ml. To the 0 µmol/ml tube, only 1500 µl Extraction Solution is added. These volumes are summarized in the following table:

Standard Dilution (µmol/ml)	1.5	1.2	1.0	0.9	0.6	0.4	0.3	0
10 µmol/ml Standard (µl)	225	180	150	135	90	60	45	0
Extraction Solution (µl)	1275	1320	1350	1365	1410	1440	1455	1500

Each tube will now contain 1500 µl of liquid.

Note:

- Some reagents have a strong odor. It is recommended the assay is conducted in an extraction cupboard. Only open the reagents in a well-ventilated area.

B. Assay Procedure

1. Set the sample, sample control, and standard microcentrifuge tubes. Each sample requires a sample tube and a sample control tube. We recommend setting up each standard, sample, and sample control in duplicate.
2. Add 500 µl of prepared standards (concentrations 1.5 µmol/ml, 1.2 µmol/ml, 1.0 µmol/ml, 0.9 µmol/ml, 0.6 µmol/ml, 0.4 µmol/ml, 0.3 µmol/ml, and 0 µmol/ml) to the standard tubes.
3. Add 500 µl of sample to the sample tubes.
4. Add 500 µl of sample to the sample control tubes.
5. Add 250 µl of Control Solution to the sample control tubes.
6. Add 250 µl of Reaction Solution to the standard tubes and the sample tubes. All tubes should now contain 750 µl of liquid.
7. Vortex all tubes for 3 minutes, then stand at room temperature for 3 minutes.
8. Set the sample, sample control, and standard wells on the microplate and record their positions.
9. Add 300 µl of standard supernatant (upper layer liquid) from the standard tubes in step 7 to the standard wells.
10. Add 300 µl of sample supernatant (upper layer liquid) from the sample tubes in step 7 to the sample wells.
11. Add 300 µl of sample control supernatant (upper layer liquid) from the sample control tubes in step 7 to the control wells.
12. Read and record absorbance at 715 nm.

C. Calculation of Results

Plot the standard curve, using the OD of the Standard Dilutions on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of NEFA in each sample well can be derived with the formula:

$$\mathbf{NEFA\ (\mu mol/g)} = \mathbf{F} \times \frac{\mathbf{OD_{Sample}} - \mathbf{b}}{\mathbf{a}} \times \left(\frac{\mathbf{V}}{\mathbf{W}}\right)$$

where:

$\mathbf{F}$	Dilution factor of the sample
$\mathbf{OD_{Sample}}$	Absorbance of the sample well at 715 nm
$\mathbf{b}$	Intercept of the standard curve
$\mathbf{a}$	Gradient of the standard curve
$\mathbf{V}$	Volume of Extraction Solution added during the tissue preparation stage (combined with sample in 1:12 ratio).
$\mathbf{W}$	Weight of tissue used during the tissue preparation stage (combined with Extraction Solution in 1:12 ratio).