Non-esterified Free Fatty Acids (NEFA) Assay Kit

Catalog No.: abx294013

Size: 100 tests

Detection range: 0.1 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 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 maximum 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 (715 nm) 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

Protocol

A. Preparation of samples and reagents

1. 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 method is intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Homogenates:** Weigh 200 mg of tissue and wash with cold PBS (0.01 M, pH 7.4). Per 0.2 g of tissue add 2.4 ml of Extraction Solution and homogenize manually, using a mechanical homogenizer, at 4°C. Mix with an orbital shaker at 4°C for 2 hours to extract the NEFA. Centrifuge the homogenate at 10,000 × g at 4°C for 10 minutes. 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. Dilute samples with Extraction Solution. 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

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. Samples should be collected and tested within 24 hours.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for tissue homogenates.

2. Reagents

- **Standards:** Label 7 tubes with 2.0 mmol/L, 1.5 mmol/L, 1.0 mmol/L, 0.5 mmol/L, 0.25 mmol/L, and 0.1 mmol/L. Add 600 µl, 450 µl, 300 µl, 150 µl, 75 µl, and 30 µl of Standard (10 mmol/L) to the 2.0 mmol/L, 1.5 mmol/L, 1.0 mmol/L, 0.5 mmol/L, 0.25 mmol/L, and 0.1 mmol/L tubes respectively, followed by 2400 µl, 2550 µl, 2700 µl, 2850 µl, 2925 µl, and 2970 µl of Extraction Solution, to prepare Standard Dilutions with concentrations 2.0 mmol/L, 1.5 mmol/L, 1.0 mmol/L, 0.5 mmol/L, 0.25 mmol/L, and 0.1 mmol/L. These volumes are summarized in the following table:

Standard Dilution (mmol/L)	**2.0**	**1.5**	**1.0**	**0.5**	**0.25**	**0.1**
10 mmol/L Standard (µl)	600	450	300	150	75	30
Extraction Solution (µl)	2400	2550	2700	2850	2925	2970

For the blank, or 0 mmol/L standard, use pure Extraction Solution. The volume of each standard will be 3000 µl.

Note:

- Sample supernatant must be clarified after sample preparation. Further centrifugation is required if the supernatant is not clarified.
- It is recommended to operate in a fume cupboard whilst carrying out steps using the Reaction Solution.

B. Assay Procedure

1. Set control, standard, and sample tubes and label accordingly. *It is recommended to prepare all tubes in duplicate.*
2. Add 1 ml of each standard to the corresponding standard tubes.
3. Add 0.5 ml of Reaction Solution to the standard tubes. *When using the Reaction Solution, operate in a fume cupboard.*
4. Add 1 ml of sample to the control tubes. Then add 0.5 ml of Control Solution to the control tubes.
5. Add 1 ml of sample to the sample tubes. Then add 0.5 ml of Reaction Solution to the sample tubes. *When using the Reaction Solution, operate in a fume cupboard.*
6. Mix using an orbital shaker for 5 minutes, then stand at room temperature for 5 minutes.
7. Set Spectrophotometer to zero using Extraction Solution..
8. Add 0.8 ml of the upper liquid layer into a 1 ml cuvette and measure the OD value at 715 nm.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) 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 following formula:

2. Tissues samples:

$$\text{NEFA}\ (\mu\text{mol/g}) = \frac{(\Delta A_{715} - b) \times V}{a \times W} \times F$$

where:

y	$OD_{Standard} - OD_{Blank}$
x	The concentration of the standard
a	The gradient of the standard curve
b	The intercept of the standard curve
ΔA_{715}	$OD_{Sample} - OD_{Control}$
V	The volume of the Extraction Solution added in the pretreatment of the tissue samples (2.4 ml)
W	The weight of the tissue (0.2 g)
F	The dilution factor of the sample

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.