Free Fatty Acid (FFA) Assay Kit

Catalog No.: abx294167

Size: 96 tests

Detection Range: 1.20 µmol/L – 100 µmol/L

Sensitivity: 1.20 µmol/L

Storage: Store all components at -20°C for up to 12 months. Store the Enzyme Reagent 1, Enzyme Reagent 2, and Substrate in the dark.

Application: For detection and quantification of Free Fatty Acid (FFA) in serum, plasma, tissue homogenates, and cell lysates.

Introduction

Free Fatty Acid (FFA) is a non-esterified fatty acid which is found in fats and oils. It is stored within adipose tissue and is released by the hydrolysis of triglycerides.

Abbexa's Free Fatty Acid (FFA) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Free Fatty Acid (FFA) content. In the presence of acyl synthase, free fatty acids produce acyl coenzyme A. Acyl coenzyme A produces hydrogen peroxide in the presence of acyl oxidase. In the presence of the enzyme and probe hydrogen peroxide reacts to produce a fluorescent substance with an optimal excitation wavelength of 535 nm and emission wavelength of 590 nm. The intensity of the fluorescence is proportional to the Free Fatty Acid (FFA) concentration, which can then be calculated.

Kit components

1. 96-well black microplate
2. Buffer Solution: 2 × 50 ml
3. Substrate: 0.12 ml
4. Enzyme Reagent 1: 2 vials
5. Enzyme Reagent 2: 2 vials
6. Scavenger: 1.2 ml
7. Standard Solution (1 mmol/L): 0.4 ml
8. Plate sealer: 2

Materials required but not provided

1. Fluorescence Microplate reader (excitation = 535 nm, emission = 590 nm)
2. Micropipette
3. Centrifuge
4. Vortex mixer
5. Incubator
6. Chloroform
7. Triton X-100
8. PBS (0.01 M, pH 7.4)

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

- **Serum** and **Plasma:** Serum and plasma samples can be tested directly.
- **Cell Lysates:** Collect the number of cells needed for the assay (initial recommendation 2 x 10^6 cells) and wash with PBS (0.01 M, pH 7.4). For every 2 x 10^6 cells, homogenize the cells in 100 µl of Buffer Solution at 4°C using an ultrasonic cell disruptor. Centrifuge at 12,000 × g for 10 minutes to remove insoluble material. Collect supernatant, keep it on ice, and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Carefully weigh at least 20 mg of the tissue sample and wash in cold PBS (0.01 M, pH 7.4). For each 20 mg of tissue, add 180 µl of Buffer Solution. Homogenize by hand, using a mechanical homogenizer at 4°C. Centrifuge the homogenate at 12,000 x g for 10 minutes to remove insoluble material. Collect the supernatant, keep on ice, and assay immediately. The protein concentration in the supernatant should be determined separately (abx097193).

  Note: To calculate Free Fatty Acid (FFA) concentration in tissue homogenates use the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately.

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 Buffer Solution, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human serum	10 - 20
Rat serum	20 - 40
Mouse plasma	10 - 30
Rabbit serum	10 - 30
10% Rat liver tissue homogenate	20 - 40
10% Mouse kidney tissue homogenate	20 - 40
10% Rat brain tissue homogenate	20 - 40
10% Rat lung tissue homogenate	20 - 40

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 cell lysates and tissue homogenates.

2. Reagents

- **Enzyme Working Solution 1:** Dissolve one vial of Enzyme Reagent 1 with 3 ml of Buffer Solution, then mix thoroughly to dissolve. The Enzyme Working Solution 1 can be stored at -20°C for up to one month in the dark.
- **Enzyme Working Solution 2:** Dissolve one vial of Enzyme Reagent 2 with 300 µl of Buffer Solution, then mix thoroughly to dissolve. The Enzyme Working Solution 2 can be stored at -20°C for up to one month in the dark.
- **Chromogenic Reagent:** For each well, prepare 50 µl of Chromogenic Reagent by thoroughly mixing 34 µl of Buffer Solution, 1 µl of Substrate, 5 µl of Enzyme Working Solution 2 and 10 µl of Scavenger. The Chromogenic Reagent should be prepared just before use and protected from light.
- **Standards:** Label 7 tubes with 100 µmol/L, 80 µmol/L, 60 µmol/L, 50 µmol/L, 40 µmol/L, 20 µmol/L, and 10 µmol/L. Add 50 µl, 40 µl, 30 µl, 25 µl, 20 µl, 10 µl, and 5 µl of Standard Solution (1 mmol/L) to the 100 µmol/L, 80 µmol/L, 60 µmol/L, 50 µmol/L, 40 µmol/L, 20 µmol/L, and 10 µmol/L tubes respectively, followed by 450 µl, 460 µl, 470 µl, 475 µl, 480 µl, 490 µl, and 495 µl of Buffer Solution, to prepare Standard Dilutions with concentrations 100 µmol/L, 80 µmol/L, 60 µmol/L, 50 µmol/L, 40 µmol/L, 20 µmol/L, and 10 µmol/L. These volumes are summarized in the following table:

Standard Dilution (µmol/L)	**10**	**20**	**40**	**50**	**60**	**80**	**100**
10 mg/ml Standard Solution (µl)	5	10	20	25	30	40	50
Buffer Solution (µl)	495	490	480	475	470	460	450

For the blank, or 0 µmol/L standard, use pure Buffer Solution. The volume of each standard will be 500 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- The Chromogenic Reagent should be kept away from light.
- Avoid repeated freezing and thawing of the Substrate, Enzyme Working Solution 1 and Enzyme Working Solution 2. It is recommended to aliquot them into smaller quantities and store at -20°C.

B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

1. Mark positions for each standard dilution and sample on the 96-well microplate.
2. Add 10 µl of each standard dilution to the standard wells.
3. Add 10 µl of samples to the sample wells.
4. Add 50 µl of Enzyme Working Solution 1 to all wells.
5. Mix thoroughly with the microplate shaker for 10 seconds, then incubate at 37°C for 10 minutes.
6. Add 50 µl of Chromogenic Reagent into all wells.
7. Mix thoroughly, then incubate at 37°C for 10 minutes.
8. Measure the fluorescence of each well with an excitation wavelength of 535 nm and an emission wavelength of 590 nm with the fluorescence microplate reader.

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 Free Fatty Acid (FFA) in each sample well can be derived with the following formulae:

1. Serum and plasma samples:

$$\text{Free Fatty Acid (FFA) (µmol/L)} = \frac{\text{f} \times (\Delta\text{F} - \text{b})}{\text{a}}$$

2. Tissue homogenate and cell lysate samples:

$$\text{Free Fatty Acid (FFA) (µmol/g protein)} = \frac{\text{f} \times (\Delta\text{F} - \text{b})}{\text{a} \times \text{C}_{\text{Protein}}}$$

where:

ΔF	Absolute F value of sample ($F_{Sample} - F_{Blank}$).
$\mathbf{f}$	Dilution factor of sample before test.
$\mathbf{C_{Protein}}$	Concentration of protein in sample (g protein/L)

Technical Support

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