Non-esterified Free Fatty Acids (NEFA) Colorimetric Assay Kit

Catalog No: E-BC-K014

Method: Colorimetric method

Specification: 96T (Can detect 92 samples without duplication)

Instrument: Microplate reader, Biochemical analyzer

Detection range: 0.01-3.0 mmol/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

Intended use

This kit can be used for detection of non-esterified free fatty acids (NEFA) content in serum, plasma, tissue homogenate, cells or cell supernatant samples.

Background

Free fatty acids, also known as non-esterified fatty acids, are derived from dietary or the metabolism of adipose tissue. In adipose tissue, hormone-sensitive lipase (HSL) decomposes triglycerides to produce glycerol and fatty acids. Circulating in the body with free fatty acids combined with plasma albumin, used as an energy source easily absorbed by muscles, brains, and other tissues and organs.

NEFA is not only the product of fat hydrolysis, but also the substrate of fat synthesis. The concentration of NEFA is related to lipid metabolism, glucose metabolism and endocrine function.

Background

NEFA and can react with coenzyme A and form acetyl-CoA under the catalysis of acetyl-CoA-synthetase (ACS). Acetyl-CoA can produce H_2O_2 when catalyzed by acetyl-CoA-oxidase (ACOD). Then H_2O_2 react with TOOS and 4-amino-antipyrine (4-APP) to generate a colored substrate under the catalysis of peroxidase (POD). The colored substrate has a maximum absorption peak at 546 nm. Measure the OD value at 546 nm and calculate the NEFA content indirectly.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Working Solution 1	20 mL × 1 vial	2-8℃ , 6 months, shading light
Reagent 2	Working Solution 2	5 mL × 1 vial	2-8℃ , 6 months, shading light
Reagent 3	1.04 mmol/L Standard	0.2 mL × 1 vial	2-8℃ , 6 months, shading light
	Microplate	96 wells	
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Biochemical analyzer (546 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents

Double distilled water, Normal saline (0.9% NaCl)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

Pre-assay preparation

▲Sample preparation

Sample requirements:

Samples (serum, plasma) can be stored at 2~8℃ for 3 days. It is recommended that the samples should be stored at -20℃ or lower temperature condition if can't detect immediately. Tissue homogenate and cell homogenate must be detected in that very day. Don't use plasma sample anticoagulated with heparin.

1. Serum or plasma:

Separate serum or plasma just in time after blood collection and avoid of hemolysis. It is recommended to detect the sample immediately. (The concentration of NEFA may increase due to the degradation of lipid.)

2. Tissue sample:

Mince the tissues to small pieces, then weighed and homogenized in normal saline on ice, the volume of normal saline (mL): the weight of the tissue (g) =9:1. The tissue homogenate is centrifuged at 2500 rpm for 10 min and take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S).

3. Cell sample:

Collect cells and treat the sample with mechanical homogenate or sonication on ice. Prepared cell homogenate does not require centrifugation. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S).

4. Cell culture supernatant:

Detect directly.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.01-3.0 mmol/L).

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	546 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don’t add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operation table

1. Main performance index

Main wavelength	546 nm	Auxiliary wavelength	600 nm
Reaction method	End-point method	Reaction temperature	37℃
Reaction direction	Up reaction (+)		

2. Operation procedure

	Blank well	Standard well	Sample well
Double distilled water (μL)	4		
Standard (μL)		4	
Sample (μL)			4
Reagent 1 (μL)	200	200	200
Mix fully and incubate at 37℃ for 5 min. Measure the OD value (A1) of each tube at 546 nm.			
Reagent 2 (μL)	50	50	50
Mix fully and incubate at 37℃ for 5 min. Measure the OD value (A2) of each tube at 546 nm wavelength. ΔA=A2-A1.			

Calculation

1. Serum (plasma) sample and other liquid sample:

$$NEFA(mmol/L) = \frac{\Delta A_{Sample} - \Delta A_{Blank}}{\Delta A_{Standard} - \Delta A_{Blank}} \times c \times f$$

2. Tissue and cells samples:

$$NEFA(mmol/gprot) = \frac{\Delta A_{Sample} - \Delta A_{Blank}}{\Delta A_{Standard} - \Delta A_{Blank}} \times c \times f \div C_{pr}$$

Note:

c: Concentration of standard.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample (gprot/L).

Performance index

1. The absorbance of blank tube: A546 nm $<$ 1.000 (optical path = 1.0 cm).
2. Linear range: 0.01-3.0 mmol/L, $r^2 \geq 0.990$.
3. Sensitivity: The ΔA value is more than 0.050 when test 1.0 mmol/L samples.
4. Accuracy: Relative deviation ≤ 15.0%. Absolute deviation ≤ 0.5 mmol/L.
5. Precision: The intra-assay CV ≤ 10% and the inter-assay CV ≤ 8%.

▲Notes

1. The kit is for scientific research only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. Do not use components from different batches of kit.
4. The validity of kit is 6 months.
5. Hemolytic sample will affect the result.
6. If the sample content is beyond linear range, please dilute the sample with normal saline before detection, and multiply the dilution multiple when calculating.
7. Choose the nearest wavelength if the instrument cannot be set to the wavelength required by this kit.
8. Personal protection measures are recommended when operating and the instructions must be strictly obeyed. The waste liquid must be treated according to the environmental protection requirement.
9. The degradation of lipid will lead to the increase of result if the sample has not been detected as soon as possible.
10. NEFA in serum has individual difference and may increase after eating.