（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K014

Specification：96T(92 samples)

Measuring instrument：Microplate reader, Biochemical analyzer

Detection range：0.01-3.0 mmol/L

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

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

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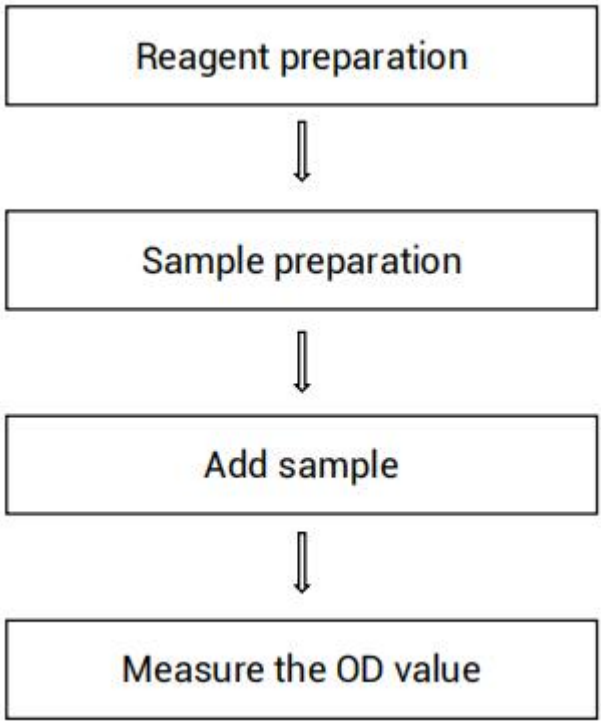
Assay summary



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.

Detection principle

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	Size (96 T)	Storage
Reagent 1	Working Solution 1	20 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 2	Working Solution 2	5 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	1.04 mmol/L Standard	0.2 mL × 1 vial	2-8°C, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Normal saline (0.9% NaCl), Double distilled water

Reagent preparation

Equilibrate all reagents to room temperature before use.

Sample preparation

Sample requirements

Samples (serum, plasma) can be stored at 2~8°C for 3 days. It is recommended that the samples should be stored at -20°C 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.

Serum and 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).

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a

dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M E-BC-K168-S, E-BC-K165-S).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 3×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 3×10^6 cells in 150 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M E-BC-K168-S, E-BC-K165-S).

Cell culture supernatant: Detect directly.

The key points of the assay

① Hemolytic sample will affect the result.

② If the sample content is beyond linear range, please dilute the sample with normal saline before detection, and multiply the dilution multiple when calculating.

③ Choose the nearest wavelength if the instrument cannot be set to the wavelength required by this kit.

④ 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.

⑤ The degradation of lipid will lead to the increase of result if the sample has not been detected as soon as possible.

⑥ NEFA in serum has individual difference and may increase after eating.

Operation table

Main performance index

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

Operation procedure

	Blank well	Standard well	Sample well
Double-diatilled water (μL)	4		
Standard (μL)		4	
Sample (μL)			4
Working Solution 1 (μL)	200	200	200
Mix fully and incubate at 37°C for 5 min. Measure the OD value (A_1) of each tube at 546 nm.			
Working Solution 2 (μL)	50	50	50
Mix fully and incubate at 37°C for 5 min. Measure the OD value (A_2) of each tube at 546 nm wavelength. $\triangle A=A_2-A_1$.			

Calculation

The sample:

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

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

2. Tissue and cell sample:

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

[Note]

ΔA_{Blank}: The change OD value of blank (A_2 - A_1).

$\Delta A_{Standard}$: The change OD value of standard (A_2 - A_1).

ΔA_{Sample}: The change OD value of sample (A_2 - A_1).

c: Concentration of standard, 1.04 mmol/L.

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, r2 ≥ 0.990.

③ Sensitivity: The △A 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%.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.