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

Catalog No：E-BC-K792-M

Specification：48T(44samples)/96T(92samples)

Measuring instrument：Microplate reader（540-550 nm）

Detection range：0.07-2 mmol/L

Elabscience® Free Fatty Acids (NEFA/FFA) Colorimetric Assay Kit (Enzyme Method)

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
Tell: 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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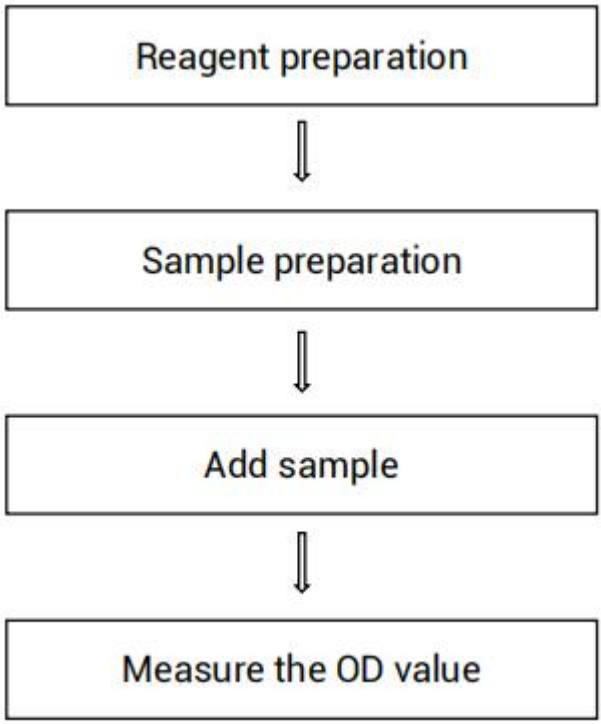
Assay summary



Intended use

This kit can be used to measure free fatty acids (NEFA/FFA) content in serum (plasma), animal tissue and cell samples.

Detection principle

Free fatty acids (NEFA/FFA) generate acyl-CoA under the action of acyl synthase, then acyl-CoA generates hydrogen peroxide under the oxidation of acyl oxidase. Hydrogen peroxide reacts in the presence of enzymes and chromogens, producing a red substance with maximum absorption at 546 nm. The amount of this red product is proportional to the content of free fatty acids.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Enzyme Diluent	12 mL ×1 vial	24 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 2	Enzyme Reagent	Powder ×2 vials	Powder ×4 vials	-20℃, 12 months, shading light
Reagent 3	Chromogenic Agent	3 mL ×1 vial	6 mL ×1 vial	2-8℃, 12 months, shading light
Reagent 4	1 mmol/L Standard	0.2 mL ×1 vial	0.2 mL ×1 vial	2-8℃, 12 months
	Microplate	48 wells	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:

Microplate reader (540-550 nm, optimum wavelength: 546 nm)

Reagents:

Normal saline (0.9% NaCl), Double distilled water

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of enzyme working solution：
Dissolve one vial of enzyme reagent with 6 mL of enzyme diluent, mix well to dissolve, incubate at 37°C for 5 min before use. Store at 2-8°C for 5 days protected from light.

③ 1 mmol/L standard incubate at 37°C for 5 min before use.

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

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).

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).

② **Dilution of sample**

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1-2
Mouse serum	4-6
Rat serum	4-6
Bovine serum	1-2
10% Rat liver tissue homogenate	1-4
10% Rat heart tissue homogenate	1-4
10% Mouse liver tissue homogenate	1
3×10^6 293T cells	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

The number of wells per assay should not exceed 20.

Operating steps

① Blank well: add 10 μL of double distilled water into the blank wells.
Standard well: add 10 μL of 1 mmol/L standard into the standard wells.
Sample well: add 10 μL of sample into the sample wells.

② Add 200 μL of enzyme working solution to each well.

③ Mix fully for 10 s and incubate at 37°C for 5 min.

⑤ Add 50 μL of chromogenic agent to each well.

⑥ Measure the OD values of each well at 546 nm recorded as A_1. Mix fully for 10 s and incubate at 37°C for 5 min. Measure the OD values of each well at 546 nm recorded as A_2. $\Delta A = A_2 - A_1$.

Calculation

The sample:

1. Serum (plasma) sample:

$$\text{NEFA/FFA content (mmol/L)} = \frac{\Delta A_{Sample} - \Delta A_{Blank}}{\Delta A_{Standard} - \Delta A_{Blank}} \times 1* \times f$$

2. Tissue and cell sample:

$$\text{NEFA/FFA content (mmol/gprot)} = \frac{\Delta A_{Sample} - \Delta A_{Blank}}{\Delta A_{Standard} - \Delta A_{Blank}} \times 1* \times f \div C_{pr}$$

[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).

1*: Concentration of standard, 1 mmol/L.

f: Dilution factor of sample before test.

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.75	1.50	3.00
%CV	6.0	5.0	2.0

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.75	1.50	3.00
%CV	3.1	6.5	8.7

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 95%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(mmol/L)	0.75	1.50	3.00
Observed Conc.(mmol/L)	0.79	1.6	3.17
Recovery rate (%)	105	107	91

Sensitivity

The analytical sensitivity of the assay is 0.07 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix Π Example Analysis

Example analysis：

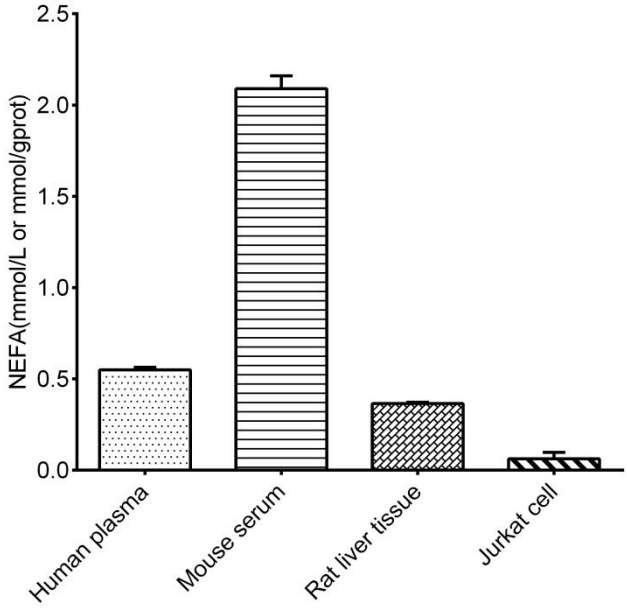
For human serum, take 10 μL of human serum and carry the assay according to the operation table. The results are as follows:

$\Delta A_{Standard}$ = 0.310 - 0.045 = 0.265, ΔA_{Sample} = 0.202 - 0.046 = 0.156,

ΔA_{Blank}= 0.114 - 0.044 = 0.070, and the calculation result is:

$$\text{NEFA/FFA content (mmol/L)} = (0.156 - 0.070) \div (0.265 - 0.070) \times 1 = 0.44 \text{ mmol/L}$$

Detect human plasma, mouse serum, 10% rat liver tissue homogenate (the concentration of protein is 11.56 gprot/L, dilute for 2 times) and Jurkat cell (the concentration of protein is 2.38 gprot/L) according to the protocol, the result is as follows：



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.