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

Catalog No：E-BC-F039

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/590 nm)

Detection range：1.20-100 μmol/L

Elabscience® Free Fatty Acids (NEFA/FFA) Fluorometric 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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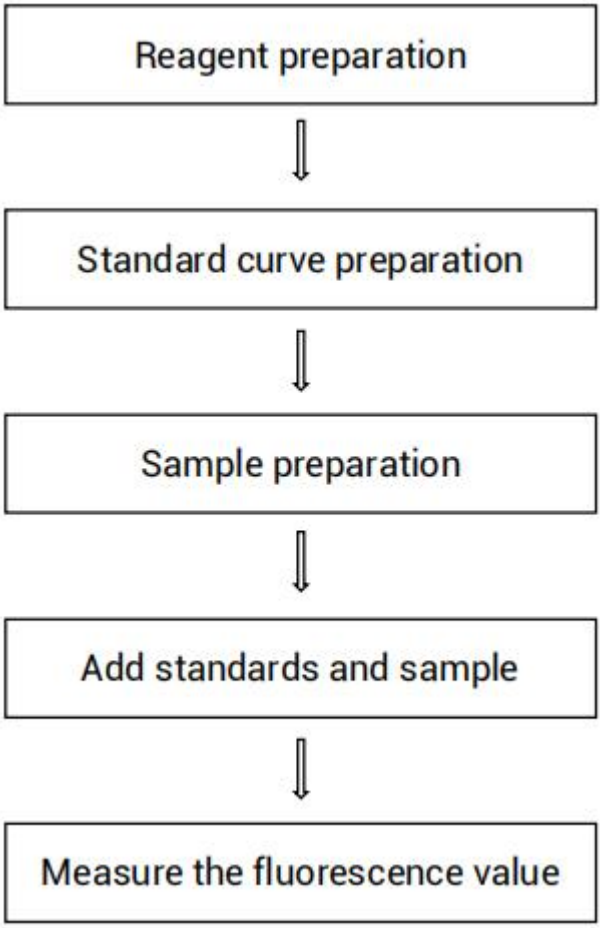
Assay summary



Intended use

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

Detection principle

Free fatty acids produce acyl coenzyme A in the presence of acyl synthase, which produces hydrogen peroxide in the presence of acyl oxidase. In the presence of the enzyme and probe, hydrogen peroxide react to produce the fluorescence substrate. The fluorescence intensity at the excitation wavelength of 535 nm and emission wavelength of 590 nm is directly proportional to the concentration of free fatty acids.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	50 mL ×1 vial	50 mL × 2 vials	-20℃, 12 months
Reagent 2	Substrate	0.06 mL × 1 vial	0.12 mL × 1 vial	-20℃, 12 months shading light
Reagent 3	Enzyme Reagent 1	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months shading light
Reagent 4	Enzyme Reagent 2	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months shading light
Reagent 5	Scavenger	0.6 mL ×1 vial	1.2 mL × 1 vial	-20℃, 12 months
Reagent 6	1 mmol/L Standard Solution	0.4 mL ×1 vial	0.4 mL × 1 vial	-20℃, 12 months
	Black 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:

Micropipette, Vortex mixer, Centrifuge, Fluorescence microplate reader (Ex/Em=535 nm/590 nm)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of enzyme application solution 1:
Dissolve one vial of enzyme reagent 1 with 3 mL of buffer solution, mix well to dissolve. Store at -20°C for 1 month protected from light.

③ The preparation of enzyme application solution 2:
Dissolve one vial of enzyme reagent 2 with 300 μL of buffer solution, mix well to dissolve. Store at -20°C for 1 month protected from light.

④ The preparation of chromogenic agent:
For each well, prepare 50 μL of chromogenic agent (mix well 34 μL of buffer solution, 1 μL of substrate, 5 μL of enzyme application solution 2 and 10 μL of scavenger). The chromogenic agent should be prepared on spot and protected from light.

⑤ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 1 mmo/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 10,

20, 40, 50, 60, 80, 100 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**10**	**20**	**40**	**50**	**60**	**80**	**100**
1 mmol/L standard (μL)	0	5	10	20	25	30	40	50
Buffer solution (μL)	500	495	490	480	475	470	460	450

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°C 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 buffer solution with a dounce homogenizer at 4°C.

④ Centrifuge at 12000×g for 10 min 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 2×10^6 cells).

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

③ Homogenize 2×10^6 cells in 100 μL buffer solution with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 12000×g for 10 min 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	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: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The chromogenic agent should be protected from light.

② Avoid repeated freezing and thawing of substrate, enzyme application solution 1 and enzyme application solution 2, it is recommended to aliquot them into smaller quantities and store at -20℃ .

Operating steps

① Standard well: Add 10 μL of standard with different concentrations.
Sample well: Add 10 μL of samples.

② Add 50 μL of enzyme application solution 1 into each wells..

③ Mix fully with microplate reader for 10 s and incubate at 37℃ for 10 min.

④ Add 50 μL chromogenic agent into each wells.

⑤ Incubate at 37℃ for 10 min.

⑥ Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 590 nm with fluorescence microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absoluted fluorescence value.

3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

$$FFA\ (\mu mol/L) = (\Delta F - b) \div a \times f$$

2. Tissue and cell sample:

$$FFA\ (\mu mol/g) = (\Delta F - b) \div a \times f \div C_{pr}$$

[Note]

ΔF: Absolute F value of sample (F_{Sample} - F_{Blank}).

f: Dilution factor of sample before tested.

C_{pr}: The 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 (μmol/L)	18.00	40.00	75.00
%CV	4.6	4.3	3.5

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 (μmol/L)	18.00	40.00	75.00
%CV	5.2	5.9	4.3

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 98%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	15	45	70
Observed Conc. (μmol/L)	15.0	45.4	70.2
Recovery rate (%)	99	97	98

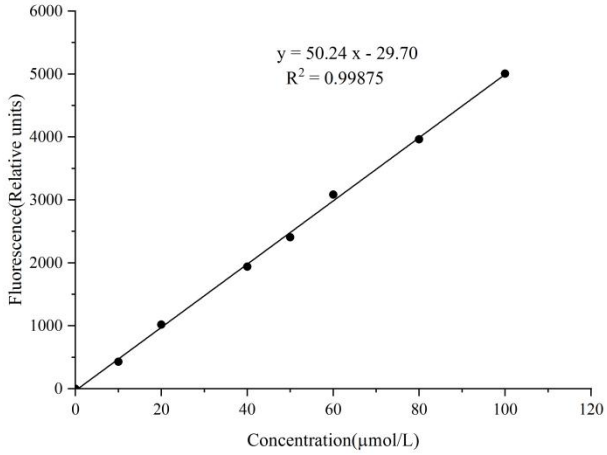
Sensitivity

The analytical sensitivity of the assay is 1.20 μmol/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.

2. Standard curve:

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (μmol/L)	0	10	20	40	50	60	80	100
Fluorescence value	333	782	1351	2273	2696	3346	4261	5264
	351	761	1374	2290	2802	3506	4349	5430
Average fluorescence value	342	772	1362	2282	2749	3426	4305	5347
Absoluted fluorescence value	0	430	1020	1940	2407	3084	3963	5005



Appendix Π Example Analysis

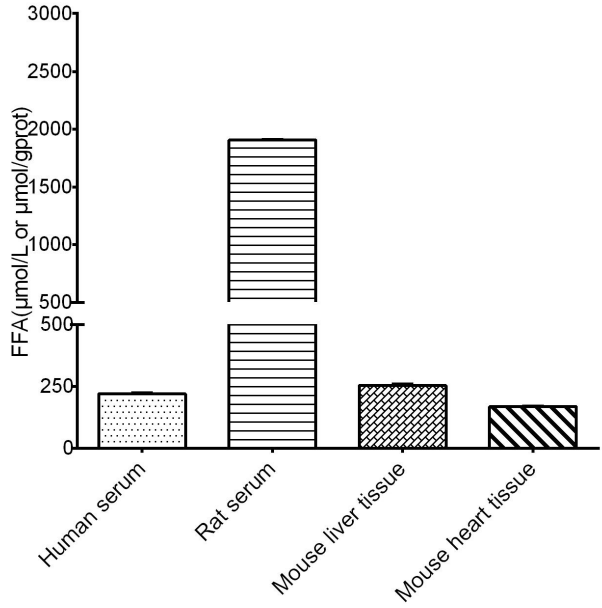
Example analysis：

For human serum, take 10 μL of human serum diluted with buffer solution for 20 times and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 50.24 x - 29.7, the average OD value of the sample is 1008, the average OD value of the blank is 492, and the calculation result is:

FFA content (μmol/L)=（1008 - 492 + 29.7）÷ 50.24 × 20 =217.23 μmol/L

Detect human serum (dilute for 20 times), rat serum (dilute for 40 times), 10% rat liver tissue homogenate (dilute for 30 times) and 10% mouse brain tissue homogenate (dilute for 30 times) 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.