PRODUCT INFORMATION & MANUAL

Free Fatty Acids (FFA) Assay Kit (Fluorometric)

NBP3-25926

For research use only.
Not for diagnostic or therapeutic procedures.

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Free Fatty Acids (FFA) Assay Kit (Fluorometric)

Catalog No. NBP3-25926

Method: Fluorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Fluorescence Microplate Reader

Sensitivity: 0.58 μmol/L

Detection range: 0.58-20 μmol/L

Average intra-assay CV (%): 4.2

Average inter-assay CV (%): 5.3

Average recovery rate (%): 96

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ 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 to measure the non-esterified free fatty acids (FFA) content in serum, plasma, animal tissue and cell 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. FFA is not only the product of fat hydrolysis, but also the substrate of fat synthesis. The concentration of FFA is related to lipid metabolism, glucose metabolism and endocrine function.

▲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	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	-20℃ , 12 months
Reagent 2	Substrate	0.12 mL × 1 vial	-20℃ , 12 months, shading light
Reagent 3	Enzyme Reagent 1	Powder ×1 vial	-20℃ , 12 months, shading light
Reagent 4	Enzyme Reagent 2	Powder ×1 vial	-20℃ , 12 months, shading light
Reagent 5	Scavenger	0.2 mL × 1 vial	-20℃ , 12 months
Reagent 6	1 mmol/L Standard Solution	0.2 mL × 1 vial	-20℃ , 12 months
Reagent 7	Extracting Solution	60 mL × 1 vial	-20℃ , 12 months, shading light
	Black Microplate	96 wells	No requirement
	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

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

Consumptive material

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

Reagents:

Double distilled water, Chloroform, Triton X-100

⚠ 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.

▲ The key points of the assay

1. If the sample is serum or plasma, it doesn't need control well.
2. If the sample is tissue or cell, the control well is essential. And it can set one control well if the dilution factor of all tissue samples is the same. Every sample needs a control well if the dilution factor of the tissue samples is different.
3. Avoid repeated freezing and thawing of reagent 2, reagent 3 application solution and reagent 4 application solution, it is recommended to aliquot them into smaller quantities and store at -20℃ .

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 3 application solution:
   Dissolve a vial of reagent 3 with 1.2 mL of reagent 1 and mix fully. The prepared reagent 3 application solution can be stored at -20℃ for 1 month with shading light.
2. Preparation of reagent 4 application solution:
   Dissolve a vial of reagent 4 with 120 μL of reagent 1 and mix fully. The prepared reagent 4 application solution can be stored at -20℃ for 1 month with shading light.
3. The preparation of chromogenic agent:
   Mix the reagent 1, reagent 2, reagent 4 application solution and reagent 5 at a ratio of 47:1:1:1. Prepare the fresh solution before use and stored with shading light.
4. The preparation of 20 μmol/L standard :
   Mix the reagent 6 and reagent 1 at a ratio of 1:49. Prepare the fresh solution before use.
5. Preparation of reagent 7 application solution (for determination of tissue samples in blank wells):

   Dilute the reagent 7 with reagent 1 according to the dilution factor of sample. For example, the tissue sample was diluted for 300 times, so dilute the reagent 7 with reagent 1 at a ratio of 1:299.
6. Preparation of control working solution (for determination of cell samples in blank wells):

   Preparation of chloroform-1% triton solution: Mix 1 mL chloroform with 0.01 mL triton (X-100) fully. Take 200 μL chloroform-1% triton solution into 2 mL EP tube and vacuum dry (50℃ , 30 min). After drying, add 200 μL reagent for resolution and mix fully for detection.

▲Sample preparation

1. Serum (plasma)

Detect directly

2. Tissue sample

Accurately weigh the tissue sample, add 9 times the volume of reagent 7 according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection.

3. Cell sample:

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (2×10^6): chloroform-1% triton solution (μL) =1: 200 for homogenizing. Vacuum dried (50℃ , 30 min). After drying, add 200 μL reagent 1 for resolution and mix fully for detection. If the liquid is turbid, centrifuge at 12000×g for 10 min before use.

▲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 (0.58-20 μmol/L).

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

Sample type	Dilution factor
Human serum	10-20
Rat serum	200-400
Mouse plasma	100-300
Rabbit serum	100-300
10% Rat liver tissue homogenate	200-400
10% Mouse kidney tissue homogenate	200-400
10% Rat brain tissue homogenate	200-400
10% Rat lung tissue homogenate	200-400

[Note]: The diluent is reagent 1.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

[Note]： A–H, standard wells; S1–S80, sample wells.

▲Detailed operation steps

The preparation of standard curve

Dilute 20 μmol/L standard solution with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 0, 3, 6, 9, 12, 15, 18, 20 μmol/L. Reference is as follows:

Number	Standard concentrations (μmol/L)	20 μmol/L standard solution (μL)	Reagent 1 (μL)
A	0	0	400
B	3	60	340
C	6	120	280
D	9	180	220
E	12	240	160
F	15	300	100
G	18	360	40
H	20	400	0

2. The measurement of samples

2.1 Serum (plasma) and tissue samples:

1) Standard well: Add 10 μL of reagent 3 application solution.
   Sample well: Add 10 μL of reagent 3 application solution.
   Control well: Add 10 μL of reagent 3 application solution.
2) Add 50 μL of standard with different concentrations into standard well.
   Add 50 μL of samples into sample well.
   Add 50 μL of reagent 7 application solution into control well.
3) Mix fully with microplate reader for 10 s and incubate at 37℃ for 30 min.
4) Add 50 μL chromogenic agent into each wells.
5) Incubate at 37℃ for 30 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.

2.2 Cell samples:

1) Standard well: Add 10 μL of reagent 3 application solution.
   Control well: Add 10 μL of reagent 3 application solution.
   Sample well: Add 10 μL of reagent 3 application solution.
2) Add 50 μL of standard with different concentrations into standard well.
   Add 50 μL of samples into sample well.
   Add 50 μL of control working solution into control well.
3) Mix fully with microplate reader for 10 s and incubate at 37℃ for 30 min.
4) Add 50 μL chromogenic agent into each wells.
5) Incubate at 37℃ for 30 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.

▲Operation table

Serum (plasma) and tissue samples:

	Standard well	Sample well	Control well
Reagent 3 application solution (μL)	10	10	10
Standard with different concentrations (μL)	50		
Samples (μL)		50	
Reagent 7 application solution			50
Mix fully with microplate reader for 10 s and incubate at 37℃ for 30 min.			
Chromogenic agent (μL)	50	50	50
Incubate at 37℃ for 30 min			
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.			

▲Notes

1. If the sample is serum or plasma, it doesn't need control well.
2. If the sample are tissue, the control well is essential. And it can set one control well if the dilution factor of all tissue samples is the same. Every sample needs a control well if the dilution factor of the tissue samples is different.

Cell samples:

	Standard well	Sample well	Control well
Reagent 3 application solution (μL)	10	10	10
Standard with different concentrations (μL)	50		
Samples (μL)		50	
Control working solution (μL)			50
Mix fully with microplate reader for 10 s and incubate at 37℃ for 30 min.			
Chromogenic agent (μL)	50	50	50
Incubate at 37℃ for 30 min			
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.			

▲Notes

1. Every sample needs a control well for cell detection.

▲Calculation

Plot the standard curve by using fluorescence value (F) of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the fluorescence value (F) of sample. The standard curve is: y = ax+b.

1. Serum (plasma) sample

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

2. Tissue sample

$$FFA\ (\mu mol/g) = (\Delta F_1 - b) \div a \times V \div m \times f$$

3. Cell sample

$$FFA\ (nmol/10^6) = (\Delta F_2 - b) \div a \times V_1 \div N \times 0.25$$

Note:

a: The slope of the standard curve.

b: The intercept of standard curve.

y: $F_{Standard} - F_{Blank}$. (F_{Blank} is the F value when the standard concentration is 0).

x: The concentration of standards.

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

ΔF_1: Absolute F value of tissue sample ($F_{Sample} - F_{Control}$).

ΔF_2: Absolute F value of cell sample ($F_{Sample} - F_{Control}$).

f: Dilution factor of sample before tested.

m: The fresh weight of tissue sample, g.

V: The volume of reagent 7 added during the pretreatment of tissue sample, L.

mL.

V_1: The volume of sample added to the reaction system, 0.05mL.

N: The number of cell sample/10^6.

1/4: The volume of sample added to the reaction system(0.05mL): total volume of samples after redissolution(0.2mL).

▲Example analysis

For human serum, take 50 μL of human serum diluted with reagent 1 for 20 times and carry the assay according to the operation table. The results are as follows:

standard curve: y = 235 x + 86, the average OD value of the sample is 1484, the average OD value of the blank is 581, and the calculation result is:

FFA content (μmol/L)= （1484 - 581 - 86） ÷ 235 × 20 =69.53 μmol/L

Appendix II References

1. Coppack S W, Persson M, Judd R L, et al. Glycerol and nonesterified fatty acid metabolism in human muscle and adipose tissue in vivo[J]. Am J Physiol, 1999, 276(2 Pt 1): E233-E240.

2. Papp M, Makara G B. The role of the lymph circulation in free fatty acid transport[J]. Experientia, 1965, 21(12): 694-694.

3. Adewuyi A A, Gruys E, van Eerdenburg F J. Non esterified fatty acids (NEFA) in dairy cattle. A review[J]. Vet Q, 2005, 27(3): 117-126.