PRODUCT INFORMATION & MANUAL

Free Fatty Acids (FFA) Assay Kit (Colorimetric)

NBP3-25931

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

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Free Fatty Acids (FFA) Assay Kit (Colorimetric)

Catalog No: NBP3-25931

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.15 mmol/L

Detection range: 0.15-1.5 mmol/L

Average intra-assay CV (%): 3.3

Average inter-assay CV (%): 5.1

Average recovery rate (%): 101

- ▲ 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 (NEFA) content in animal tissue 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.

▲Detection principle

Under the condition of weak acidity, NEFA react with nantokite to form copper soap, which has a specific absorption peak at 715nm. The content of NEFA can be calculated indirectly by measuring the OD value at 715 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extracting Solution	60 mL × 2 vials	2-8℃，12 months
Reagent 2	10 mmol/L Palmitic Acid Standard	1 mL × 2 vials	2-8℃，12 months
Reagent 3	Control Solution	12 mL ×1 vial	2-8℃，12 months
Reagent 4	Reaction Solution	20 mL × 1 vial	2-8℃，12 months
	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

Microplate reader, Vortex instrument, Micropipettor (20-200 μL, 100-1000 μL), Multichannel transferpettor (300 μL).

Consumptive material

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

⚠ 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. The samples should be fresh collected and detected within 24 hours.
2. The supernatant after centrifugation must be clarified for the pretreatment of tissue samples. Otherwise take the turbid supernatant to another centrifuge tube and centrifuge again.
3. The reagent has a pungent smell. Please operate in the draught cupboard.

Pre-assay preparation

▲ Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

Sample requirements

The samples should be fresh collected and detected within 24 hours.

▲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 (0.15-1.5 mmol/L).

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

Sample type	Dilution factor
Rat liver tissue homogenate	1
Rat heart tissue homogenate	1
Rat kidney tissue homogenate	1
Mouse liver tissue homogenate	1

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	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note: A-H, standard wells; S1-S40, sample wells; S1'-S40', control wells.

▲Detailed operation steps

The preparation of standard curve

Dilute 10 mmol/L standard solution with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 0, 0.3, 0.4, 0.6, 0.9, 1, 1.2, 1.5 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	10 mmol/L standard solution (μL)	Reagent 1 (μL)
A	0	0	1500
B	0.3	45	1455
C	0.4	60	1440
D	0.6	90	1410
E	0.9	135	1365
F	1.0	150	1350
G	1.2	180	1320
H	1.5	225	1275

The measurement of samples

(1) Standard tube: Add 0.5 mL of standard with different concentrations and add 0.25 mL of reagent 4.

Control tube: Take 0.5 mL of the supernatant of sample and add 0.25 mL of of reagent 3.

Sample tube: Take 0.5 mL of the supernatant of sample and add 0.25 mL of reagent 4.

(2) Oscillate for 3 min and stand at room temperature for 3 min.

(3) Take 0.3 mL of the upper layer liquid to micro-plate and measure the OD value at 715 nm with microplate reader.

▲Summary operation table

	Standard tube	Sample tube	Control tube
Standard with different concentrations (mL)	0.5		
Sample (mL)		0.5	0.5
Reagent 3 (mL)			0.25
Reagent 4 (mL)	0.25	0.25	
Oscillate for 3 min, stand at room temperature for 3 min. Take 0.3 mL of the upper layer liquid to 96-wells microplate and measure the OD value at 715 nm with Microplate reader.			

▲Calculation

Plot the standard curve by using OD value 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 OD value of sample.

The standard curve is: $y= ax + b$.

For tissue sample:

$$NEFA\ (\mu mol/g) = (\Delta A_{715} - b) \div a \times (V_1 \div m) \times f$$

Note:

y: The absolute OD value of standard ($OD_{Standard} - OD_{Blank}$).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{715}: absolute OD value of sample.

m: the fresh weight of tissue sample, 0.1 g.

V_1: the volume of Reagent 1 added during the pretreatment of tissue sample, 1.2 mL.

f: Dilution factor of sample before tested.

Appendix I Data

▲Example analysis

For rat liver tissue, take 0.1 g of rat liver tissue, add 1.2 mL reagent 1, oscillate at 4℃ for 2 hours to extract the NEFA, centrifuge at 10000×g for 10 min, dilute the supernatant with reagent 1 for 3 times, then carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.09174 x – 0.0026, the average OD value of the sample tube is 0.108, the average OD value of the control tube is 0.049, and the calculation result is:

NEFA content (μmol/g)=(0.108-0.049+0.0026)÷0.09174×(12÷0.1)×3

=24.17 μmol/g

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Tissue sample

Wash the tissue with PBS (2~8℃ , 0.01M, pH=7.4), then remove the water on surface of the tissue with absorbent paper. Weigh and mince the tissue, then add Reagent 1 according to the ratio of Weight (g): Reagent 1 (mL) =1: 12, homogenize the sample and oscillate at 4℃ for 2 hours to extract the NEFA. Centrifuge the sample at 10000 g for 10 min at 4℃ and take the supernatant for detection.

Note:

1. Homogenized medium: Reagent 1.
2. Homogenized method:
   (1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

Appendix III 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.