CheKine™ Free Fatty Acid (FFA/NEFA) Content Assay Kit (Enzymatic Method)

Cat #: KTB2231 Size: 48 T/48 S 96 T/96 S

	Free Fatty Acid (FFA/NEFA) Content Assay Kit (Enzymatic Method)		
REF	**Cat #:** KTB2231	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum (plasma), animal and plant tissues, cells		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Non-esterified fatty acids (NEFA) are the primary product of lipolysis in adipose tissue, and their serum levels directly reflect the body's lipid metabolic status. Elevated serum NEFA levels are closely associated with insulin resistance, type II diabetes, obesity, and cardiovascular diseases; conversely, reduced levels indicate impaired endocrine function. Therefore, NEFA testing is an important indicator for screening lipid metabolic disorders and monitoring disease progression.

This assay kit employs an enzyme-coupled colorimetric method. Free fatty acids in the sample are converted into acetyl-CoA by acyl-CoA synthetase (ACS), which is then oxidized by acyl-CoA oxidase (ACOD) to produce hydrogen peroxide. Under catalysis by peroxidase (POD), hydrogen peroxide reacts with a chromogenic substrate to form a purple quinone dye. The absorbance of this dye at 546 nm is directly proportional to the concentration of free fatty acids in the sample.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	12 mL	24 mL	-20℃, protected from light
Reagent Ⅱ	3 mL	6 mL	-20℃, protected from light
Reagent Ⅲ	0.5 mL	1 mL	-20℃, protected from light
Standard	0.5 mL	0.5 mL	-20℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

·Microplate reader capable of measuring absorbance at 546 nm
·96-well plate, precision pipettes, disposable pipette tips
·Incubator, refrigerated centrifuge
·Deionized water, PBS (1×, pH 7.4)
·Homogenizer, ultrasound disintegrator

Reagent Preparation

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃, protected from light.

Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃, protected from light.

Reagent Ⅲ: Ready to use as supplied. Equilibrate to room temperature before use. The reagent is volatile, seal tightly after each use. Store at -20℃, protected from light.

1 mmol/L Standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.

Sample Preparation

Note: We recommend that you use fresh samples. Serum (plasma) samples should be tested as soon as possible after collection. If they cannot be tested on the same day, they should be stored at -80℃ and used within a month. Samples should be extracted as fresh as possible. If they cannot be extracted on the same day, intact cells or aliquoted tissue blocks should be stored at -80℃ and used within a month. When preparing to conduct this experiment, take the samples out of -80℃ and thaw them on ice. However, please note that this may affect the stability of the samples and the experimental results may be lower than expected. The supernatant after sample homogenization should be tested as soon as possible to ensure the accuracy of the measurement values.

1. Serum (plasma) : After collection, serum or plasma should be separated promptly to avoid hemolysis
2. Animal or plant tissues: Weigh 0.1 g tissues, add 0.9 mL PBS (1×, pH 7.4) and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells with cold PBS (1×, pH 7.4), discard the supernatant after centrifugation; add 0.2 mL PBS (1×, pH 7.4) to ultrasonically disrupt the cells 5 min on ice (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Assay Procedure

1. Preheat the microplate reader for more than 30 min, and adjust the wavelength to 546 nm.
2. Working Reagent Preparation: Prepare immediately before use. For each well, prepare 52 μL Working Reagent by mixing 48 μL Reagent Ⅱ and 4 μL Reagent Ⅲ.
3. Sample measurement. (The standard well and the blank well only need to be tested once. Operate in 96-well plate as follows)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Deionized water	10	0	0
1 mmol/L Standard	0	10	0
Sample	0	0	10
Reagent Ⅰ	200	200	200
Mix well, incubate at 37℃ for 5 min.The absorbance value is measured at 546 nm. Record as A_{1Blank}, $A_{1Standard}$, and A_{1Test}.			
Working Reagent	50	50	50

4. Mix well, incubate at 37℃ for 5 min. The absorbance value is measured at 546 nm. Record as A_{2Blank}, $A_{2Standard}$, and A_{2Test}. Finally calculate $\Delta A_{Blank}=A_{2Blank}-A_{1Blank}$, $\Delta A_{Standard}= A_{2Standard}- A_{1Standard}$, $\Delta A_{Test}=A_{2Test}- A_{1Test}$.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Calculated by serum (plasma) samples:

NEFA (mmol/L)=$(\Delta A_{Test}-\Delta A_{Blank})\div(\Delta A_{Standard}-\Delta A_{Blank})\times C_{Standard}\times n=$**$(\Delta A_{Test}-\Delta A_{Blank})\div(\Delta A_{Standard}-\Delta A_{Blank})\times n$**

2. Calculated by weight of samples:

NEFA (μmol/g)=(ΔA_{Test}-ΔA_{Blank})÷($\Delta A_{Standard}$-ΔA_{Blank})×$C_{Standard}$÷W×$V_{Extraction}$×n=**(ΔA_{Test}-ΔA_{Blank})÷($\Delta A_{Standard}$-ΔA_{Blank})×0.9÷W×n**

3. Calculated by cell number:

NEFA (μmol/10^6)=(ΔA_{Test}-ΔA_{Blank})÷($\Delta A_{Standard}$-ΔA_{Blank})×$C_{Standard}$÷N×$V_{Extraction}$×n=**(ΔA_{Test}-ΔA_{Blank})÷($\Delta A_{Standard}$-ΔA_{Blank})×0.2÷N×n**

4. Calculated by protein concentration:

NEFA (mmol/g prot)=(ΔA_{Test}-ΔA_{Blank})÷($\Delta A_{Standard}$-ΔA_{Blank})×$C_{Standard}$÷C_{pr}×n=**(ΔA_{Test}-ΔA_{Blank})÷($\Delta A_{Standard}$-ΔA_{Blank})÷Cpr×n**

Where: $C_{standard}$: concentration of the standard , 1 mmol/L = 1 μmol/mL; $V_{Extraction}$: the total volume of the extraction solution PBS (1×, pH 7.4) , for tissue sample is 0.9 mL and for cell sample is 0.2 mL; W: sample weight, g; N: total number of cells, with the unit of 10^6 (e.g., total number of cells: 5×10^6, N=5); Cpr; sample protein concentration, g/L; n: dilution factor.

Precautions

1. What if the obtained ΔA_{Test} is too high or too low?

If ΔA_{Test} is higher than 0.56, dilute the sample appropriately with PBS (1×, pH 7.4) and repeat the assay. If ΔA_{Test} is lower than 0.01, increase the sample amount (animal or plant tissue weight) or decrease the PBS (1×, pH 7.4) volume, then repeat the assay.

2. What if the sample volume is too small?

It is recommended to select 2-3 samples for dilution and testing before the formal experiment. If the ΔA_{Test} of the diluted samples is higher than 0.01, then the sample volume can be reduced by dilution.

Typical Data

Examples:

1. Test 0.1g mouse liver, following the above protocol and measured with the 96-well microplate:

$\Delta A_{Blank}=A_{2Blank}-A_{1Blank}$=0.067-0.043=0.024, $\Delta A_{Standard}= A_{2Standard}- A_{1Standard}$=0.323-0.043=0.280,

$\Delta A_{Test}=A_{2Test}- A_{1Test}$=0.142-0.083=0.059.

2. Calculated by weight of samples:

NEFA (μmol/g)=(ΔA_{Test}-ΔA_{Blank})÷($\Delta A_{Standard}$-ΔA_{Blank})×0.9÷W×n=(0.059-0.024)÷(0.280-0.024)×0.9÷0.1×1=1.23 μmol/g.

Recommended Products

Catalog No.	Product Name
KTB2200	CheKine™ Micro Triglyceride (TG) Assay Kit
KTB2220	CheKine™ Micro Total Cholesterol (TC) Assay Kit
KTB2260	CheKine™ Micro Low Density Lipoprotein Cholesterol (LDL-C) Assay Kit
KTB2250	CheKine™ Micro High Density Lipoprotein Cholesterol (HDL-C) Assay Kit
KTB2302	CheKine™ Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB2303	CheKine™ Aspartate Aminotransferase (AST/GOT) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.