CheKine™ Micro Free Fatty Acid (FFA) Assay Kit

Cat #: KTB2230 Size: 48 T/96 T

	Micro Free Fatty Acid (FFA) Assay Kit		
REF	**Cat #:** KTB2230	LOT	**Lot #:** Refer to product label
	Detection range: 0.0313-2 mM		**Sensitivity:** 0.0156 mM
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Free Fatty Acids (FFA), also known as non-esterified fatty acids, are not only the product of fat hydrolysis, but also the substrate of fat synthesis, circulating in plasma combined with albumin. The concentration of FFA in serum is related to lipid metabolism, carbohydrate metabolism and endocrine function. The concentration of FFA will increase due to diabetes, severe liver dysfunction, hyperthyroidism and other diseases. CheKine™ Micro Free Fatty Acid (FFA) Assay Kit provides a convenient tool for detection of FFA in Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria. The principle is that the FFA combines with copper ions to form copper salt of fatty acid, which is soluble in chloroform. The content of free fatty acid can be calculated by determining the content of copper ion with copper reagent method.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Cu Reagent	5.375 mL	10.75 mL	4℃, protected from light
Chromogen	15 mL	30 mL	4℃, protected from light
Standard (16.41 mg Palmitic Acid)	1	1	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 550 nm
- Incubator, ice maker, refrigerated centrifuge
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Homogenizer (for tissue samples)
- Glass bottle (for preparation of extraction buffer)
- N-heptane, anhydrous methanol, chloroform

Reagent Preparation

Extraction Buffer: Prepare yourself. Take a glass bottle and prepare Extraction Buffer, according to the ratio of chloroform: N-heptane: Anhydrous methanol =28:21:1. The mixture was covered tightly and stored at 4℃, protected from light.

Cu Reagent: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Chromogen: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Standard: Add 1 mL Extraction Buffer to dissolve before use. The concentration is 64 mM. This solution can be stored in glass bottle, cover tightly, at 4℃, protected from light.

Standard curve setting: dilute 64 mM standard with Extraction Buffer as shown in the table below.

Num.	Volume of Standard	Volume of Extraction Buffer (μL)	The Concentration of Standard（mM）
Std.1	20 μL of 64 mM	620	2
Std.2	100 μL of Std.1 (2 mM)	100	1
Std.3	100 μL of Std.2 (1 mM)	100	0.5
Std.4	100 μL of Std.3 (0.5 mM)	100	0.25
Std.5	100 μL of Std.4 (0.25 mM)	100	0.125
Std.6	100 μL of Std.5 (0.125 mM)	100	0.0625
Std.7	100 μL of Std.6 (0.0625 mM)	100	0.0313

Note：Always prepare fresh standards per use.

Sample Preparation

Note: Fresh samples are recommended. If not assayed immediately, samples can be stored at -80℃ for 6 months.

1. Animal tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 rpm for 10 min at 4℃. Use supernatant for assay.
2. Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and mash. Ultrasonic break on ice 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 rpm for 10 min at 4℃. Use supernatant for assay.
3. Cells or bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria on ice 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 rpm for 10 min at 4℃. Use supernatant for assay.
4. Liquid samples such as serum: Tested directly.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 550 nm, visible spectrophotometer was returned to zero with deionized water.
2. Add the following reagents respectively into each EP tube:

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)
Extraction Buffer	240	200	200
Stds.	0	40	0
Sample	0	0	40
Mix well, cover tightly and place the mixture on the vortex mixer for 30 s at medium speed			
Cu Reagent	80	80	80
Mix well, cover tightly and place the mixture on the vortex mixer for 30 s at medium speed, incubate at room temperature (25℃) for 20 min. Centrifuge at 2,000 g for 5 min at room temperature (25℃)			
Superstratum Solution	50	50	50

Chromogen	200	200	200

3. Incubate at room temperature (25℃) for 5 min. Take out 200 μL to a 96-well plate or microglass cuvette. Then reading the values at 550 nm. Finally, calculate $\Delta A_{Test} = A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$. (Only one blank well needs to be detected). Be sure to finish the read within 30 min after color development.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If A_{Test} is greater than detection range of microplate reader, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor.

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. Drawing of standard curve

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve. Substitute the ΔA_{Test} into the equation to obtain the y value (mM).

2. Calculate the content of FFA

(1) By sample fresh weight

FFA (μmol/g)=y÷(W÷$V_{Extraction}$)×n**=y÷W×n**

(2) Calculated by cells or bacteria number

FFA (μmol/10^4)=y÷(Cells or bacteria number÷$V_{Extraction}$)×n=y÷500**=0.002×y×n**

(3) Calculated by liquid volume

FFA (μmol/L)**=1,000×y×n**

Where: $V_{Extraction}$: Extraction Buffer volume added, 1 mL; W: sample weight, g; n: dilution multiple of sample further dilution; 500: Total number of bacteria or cells, 5×10^6; 1,000: 1 L=1,000 mL。

Typical Data

Typical standard curve

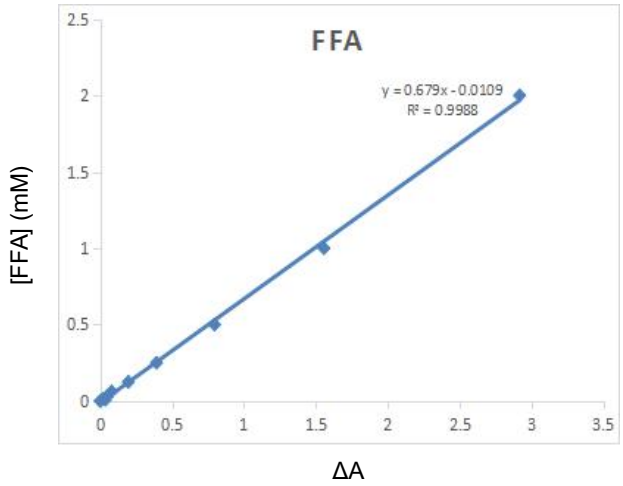

Figure 1. Standard curve of FFA in 96-well plate assay–data provided for demonstration purposes only. A new standard curve must be generated for each assay

Examples

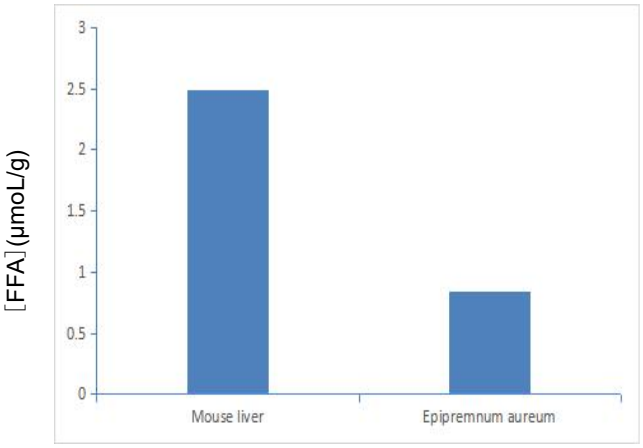

Figure 2. FFA content in mouse liver and arabidopsis respectively. Assays were performed following kit protocol

Recommended Products

Catalog No.	Product Name
KTB2200	CheKine™ Micro Triglyceride (TG) Assay Kit
KTB2210	CheKine™ Micro Free Cholesterol (FC) Assay Kit
KTB2220	CheKine™ Micro Total Cholesterol (TC) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.