Free Fatty Acids (FFA) Assay Kit((Microplate Method))

Catalogue No.: K134

Size: 48T(48S)/96T(96S)

Range: 0.0313-2mM

Sensitivity: 0.0156mM

Kit component:

Reagents	48T	96T	Storage
Cu Reagent	6 mL	12 mL	2-8°C protected from light
Chromogen	15 mL	30 mL	2-8°C protected from light
Standard (16.41 mg Palmitic Acid)	Powder×1 vial	Powder×1 vial	2-8°C protected from light

Storage:

The kit should be stored at 2-8℃ shading light for one year.

Application:

This kit can be used to measure the content of free fatty acids in biological samples, including serum (plasma), animal and plant tissues, cells and bacteria.

Principle of the Assay:

Free fatty acids (FFA), also known as non-esterified fatty acids, are both the products of fat hydrolysis and the substrates for fat synthesis, and they circulate in the plasma bound to albumin. The concentration of FFA in serum is related to lipid metabolism, glucose metabolism, and endocrine function. The concentration of FFA will increase due to diseases such as diabetes, severe liver disorders, and hyperthyroidism.

The principle of Free Fatty Acids (FFA) Assay Kit((Microplate Method)) is that FFA combines with copper ions to form copper fatty acid salts, which dissolve in chloroform. The content of copper ions is determined by the copper reagent method, and the content of free fatty acids can be calculated accordingly.

Materials Not Supplied:

microplate reader or visible spectrophotometer(capable of measuring absorbance at 550nm), incubator, ice maker, low-temperature centrifuge

96-well plates or micro glass cuvettes, adjustable pipettes and pipette tips, homogenizer (for tissue samples), glass bottles (for preparing extraction solution)

n-heptane, anhydrous methanol, chloroform (trichloromethane)

Assay Procedure (For reference):

Reagent preparation

Extraction Buffer（self-prepared）： Take a glass bottle, prepare it with a ratio of chloroform: n-heptane: anhydrous methanol = 28:21:1. Seal it tightly and mix well. Store it at 4℃ protected from light.

Cu Reagent： Ready-to-use; Bring it to room temperature and mix well before use; Store it at 4℃ protected from light.

Chromogen： Ready-to-use; Bring it to room temperature before use; Store it at 4℃ protected from light.

Standard： Prepare just before use. Add 1 mL of Extraction Buffer and thoroughly dissolve to obtain 64 mM Standard. Any unused dissolved Standard can be stored in a glass bottle with a tight seal at 4°C away from light for up to one month.

Setting of the standard curve: Dilute the 64 mM standard with the Extraction Buffer for the next step, as shown in the figure.

Number	Volume of the standard sample	Volume of Extraction Buffer（μL）	Concentration of the standard sample（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: For each experiment, please use the freshly diluted standard samples.

➤ Sample preparation

Note: It is recommended to use fresh samples. If the experiment is not conducted immediately, the samples can be stored at -80℃ for 6 months.

1. Animal tissue: Weigh approximately 0.1g of the sample, add 1 mL of Extraction Buffer, homogenize on ice, centrifuge at 8,000 rpm for 10 minutes at 4℃, then take the supernatant and place it on ice for measurement.

2. Plant tissue: Weigh approximately 0.1g of the sample, add 1mL of Extraction Buffer, and crush. Place in an ice bath and subject to ultrasonic disruption for 5 minutes (power 20% or 200 W, ultrasonic 3 seconds, interval 7 seconds, repeat 30 times). Centrifuge at 8,000 rpm for 10 minutes at 4°C. Take the supernatant and keep it on ice for testing.

3. Cells or bacteria: Collect 5 million cells or bacteria into a centrifuge tube, wash the cells with cold PBS, centrifuge and discard the supernatant, add 1 mL of Extraction Buffer, perform ice bath ultrasonic disruption for 5 minutes (power 20% or 200 W, ultrasonic 3 seconds, interval 7 seconds, repeat 30 times), centrifuge at 8,000 rpm for 10 minutes at 4°C, take the supernatant and keep it on ice for testing.

4. Serum and plasma: detect directly.

➤ Experimental procedures

1. The microplate reader or visible spectrophotometer is preheated for more than 30min, the wavelength is adjusted to 550nm, and the distilled water is adjusted to zero.

2. Add samples and test (add the following reagents sequentially to the Eppendorf tube):

Reagent	Blank tube（μL）	Standard tube（μL）	Test tube（μL）
Extraction Buffer	240	200	200
Standard with different concentrations	0	40	
Sample			40
Cap the bottle tightly after mixing and place it on a vortex mixer to vortex at medium speed for 30 seconds.			
Cu Reagent	80	80	80
Cap the bottle tightly after mixing and place it on a vortex mixer to vortex at medium speed for 30 seconds, place at room temperature (25°C) for 20 minutes; centrifuge at 2,000g for 5 minutes at room temperature (25°C).			
Take the upper layer solution	50	50	50
Chromogen	200	200	200

3. Place at room temperature (25°C) for 5 minutes. Take 200 μL from each tube and add it to the corresponding wells of a 96-well plate or a micro glass cuvette. Measure the absorbance at 550 nm and calculate ΔA test = A test - A blank, ΔA standard = A standard - A blank (the blank tube only needs to be prepared once). The color development must be completed within 30 minutes.

Note: Before the formal test, 2-3 samples with significant differences should be selected for pre-test. If the A measurement is greater than the detection range of the microplate reader, the sample can be further diluted with Extraction Buffer, and the final result should be multiplied by the dilution factor.

Calculation:

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. Plot the standard curve

Plot a standard curve with the standard solution concentration as the y-axis and ΔA standard as the x-axis (it is more convenient to calculate the results when the concentration is on the y-axis). Substitute ΔA test into the standard curve formula to calculate y (mM).

2. Calculation of FFA content in the sample

(1) Calculated by sample weight

FFA content(μmol/g)=y×Vextract÷W×n=**y÷W×n**

(2) Calculated by cells or bacteria number

FFA content(μmol/10^4)=y÷(The number of cells or bacteria÷Vextract)×n=y÷500×n=**0.002×y×n**

(3) Calculated by liquid volume

FFA content(μmol/L)=**1,000×y×n**

Note:

Vextract：The volume of the extracted liquid added, 1mL；

W：sample weight, g；

n：The dilution factor of the sample；

500：Total number of bacteria or cells, 5 million；

1,000：Unit conversion factor, 1L=1,000mL。

Notes:

1. This product is for scientific research use by professionals only.

2. Please pay attention to safety precautions and follow the laboratory reagent operation norms. For your safety and health, please wear a lab coat and disposable gloves during the operation.