Non-Esterified Fatty Acids Content Assay Kit

Cat. No. KITG-080 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Free fatty acids (FFAs), also known as non-esterified fatty acids (NEFAs), are mainly generated by the breakdown of neutral fats. They are intermediate products of lipid metabolism, participate in cell proliferation, inflammatory responses, hormone regulation, and other processes, and can also function as signaling molecules with multiple physiological roles. Free fatty acids are closely associated with lipid metabolism, glucose metabolism, and endocrine function. Their content is an important physiological and biochemical indicator, may serve as an auxiliary parameter for disease evaluation and diagnosis, and can also reflect quality changes during food storage. Free fatty acids bind copper ions to form copper soaps. The copper ions then react with the chromogenic reagent to form a purplish-red complex with a characteristic absorption peak at 550 nm. The free fatty acid content can therefore be quantitatively determined from the change in absorbance.
Description	Method: Visible spectrophotometry. Pack Size: 70 tests / 50 samples. Storage Temperature: 2-8°C. For Research Use Only. Not for Use in Diagnostic Procedures.
Form	Kit
Applications	Quantitative determination of non-esterified fatty acid/free fatty acid content in tissue samples, serum, culture medium, and other liquid samples.
Notes	Instructions for Use Required Instruments and Reagents: Visible spectrophotometer, 1 mL glass cuvette with a 10 mm optical path, mortar/homogenizer, adjustable pipette, benchtop centrifuge, thermostatic water bath, n-heptane, anhydrous

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methanol, absolute ethanol, chloroform, and distilled water. 1. Sample Treatment (adjust the sample amount and ratio appropriately according to preliminary test results) 1) Tissue: Rinse the tissue thoroughly with physiological saline and blot the surface moisture dry with absorbent paper. Process the sample at a tissue mass (g) to Extraction Solution volume (mL) ratio of 1:(5-10). It is recommended to weigh 0.1 g tissue and add 1 mL Extraction Solution. Homogenize thoroughly, centrifuge at 8,000 rpm for 10 min at 4°C, collect the supernatant as the test sample, and keep it on ice until measurement. 2) Serum, culture medium, and other liquid samples: Allow the blood sample to stand at room temperature for 1 h, centrifuge at 3,500 rpm for 15 min at 4°C, collect the upper serum layer, and store it at 4°C until measurement. Culture medium and other liquid samples may be measured directly or after appropriate dilution. 2. Assay Procedure 1) Preheat the spectrophotometer for at least 30 min, set the wavelength to 550 nm, and zero the instrument with absolute ethanol. 2) Preheat Reagent 1 at 37°C for at least 30 min before the assay. 3) Add the following reagents sequentially to centrifuge tubes: Test Tube: 50 μ test sample + 500 μ Extraction Reagent + 200 μ Reagent 1. Control Tube: 50 μ distilled water + 500 μ Extraction Reagent + 200 μ Reagent 1. Standard Tube: 50 μ standard dilution + 500 μ Extraction Reagent + 200 μ Reagent 1. Blank Tube: 50 μ chloroform + 500 μ Extraction Reagent + 200 μ Reagent 1. Shake thoroughly for 10 min, then centrifuge at 3,000 rpm for 10 min at room temperature. Transfer 200 μ of the upper-layer solution from each tube to a new tube, add 800 μ Reagent 2, shake thoroughly for 2 min, and allow to stand at room temperature for 15 min. Absorbance Measurement: Complete the measurement within 30 min. Measure absorbance at 550 nm and record the values as A_test, A_control, A_standard, and A_blank. Calculate ΔA_test = A_test - A_control and ΔA_standard = A_standard - A_blank. The blank and control tubes only need to be measured 1-2 times. Standard Curve: Use 1.0, 0.8, 0.4, 0.2, 0.1, and 0.05 μmol/mL as the x-axis values and the corresponding ΔA_standard values as the y-axis values. Plot the standard curve and obtain the linear equation y = kx + b. Substitute ΔA_test into the equation to obtain x in μmol/mL. 3. Non-Esterified Fatty Acid Content

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	Calculations 1) Based on tissue protein content: FFA content (μmol/mg prot) = (x × V_total)/(Cpr × V_total) = x/Cpr. 2) Based on tissue sample mass: FFA content (μmol/g) = (x × V_total)/W. 3) Based on bacterial or cell number: FFA content (μmol/10^4 cells) = (x × V_total)/(bacterial or cell count). 4) Based on liquid sample volume: FFA content (μmol/L) = 1000 × D × x. Where V_total is the total volume of the test sample, 1 mL; Cpr is the sample protein concentration in mg/mL; W is the sample mass in g; bacterial or cell count is expressed in units of 10^4; D is the dilution factor of the liquid sample; and 1000 is the unit-conversion factor, where 1 L = 1000 mL. 4. Precautions 1) Prepare Reagent 2 as late as possible. It may be prepared when the procedure reaches the step of adding Reagent 1. 2) Ensure that the shaking frequency and shaking time are consistent for every tube. 3) Complete the measurement within 30 min. After measurement, seal the tubes before disposal. 4) Most reagents used in the assay are organic solvents. Repeated aspiration with the same pipette tip may cause inaccurate volumes; replace the tip after dispensing each liquid. 5) If the measured absorbance is outside the linear absorbance range of the standards, dilute the test sample appropriately and repeat the measurement when the value is above the highest standard. When the value is below the lowest standard, appropriately increase the sample amount and repeat the measurement. Modify the calculation accordingly. 6) To ensure accurate results and avoid reagent loss, carefully read the instructions before measurement, confirm that reagent storage and preparation are adequate, verify that the operating steps are clear, and perform a preliminary test using 2-3 samples expected to show substantial differences. If any problem occurs during the procedure, seek technical assistance promptly.
Storage	Kit Components and Storage: Extraction Solution: liquid, 60 mL × 1 bottle; store at 4°C. Reagent 1: liquid, 15 mL × 1 bottle; store at 4°C protected from light. Reagent 2: powder, 3 bottles; store at 4°C protected from light. Before use, add 30 mL absolute ethanol to each bottle and dissolve completely. Prepare immediately before use; after

	preparation, it may be stored at 4°C for one week. Standard: powder, 1 vial containing 10 mg palmitic acid standard; store at room temperature. Before use, add 780 µl chloroform and dissolve completely to obtain a 50 µmol/mL palmitic acid standard solution. Store the prepared standard solution sealed at 4°C.
Reconstitution	Reagent and Standard Preparation: 1. Reagent 2: Add 30 mL absolute ethanol to each bottle and dissolve completely. Prepare as late as possible before use. The prepared reagent may be stored at 4°C for one week. 2. Palmitic Acid Standard Solution: Add 780 µl chloroform to the 10 mg palmitic acid standard and dissolve completely to obtain a 50 µmol/mL standard solution. Store sealed at 4°C. 3. Standard Dilutions: Dilute the 50 µmol/mL palmitic acid standard solution with chloroform to prepare 1.0, 0.8, 0.4, 0.2, 0.1, and 0.05 µmol/mL standards. Prepare the dilution sequence as follows: Standard A: 200 µl of 50 µmol/mL standard + 800 µl chloroform; final concentration 10 µmol/mL. Standard 1: 100 µl of 10 µmol/mL standard + 900 µl chloroform; final concentration 1.0 µmol/mL. Standard 2: 80 µl of 10 µmol/mL standard + 920 µl chloroform; final concentration 0.8 µmol/mL. Standard 3: 500 µl of 0.8 µmol/mL standard + 500 µl chloroform; final concentration 0.4 µmol/mL. Standard 4: 500 µl of 0.4 µmol/mL standard + 500 µl chloroform; final concentration 0.2 µmol/mL. Standard 5: 500 µl of 0.2 µmol/mL standard + 500 µl chloroform; final concentration 0.1 µmol/mL. Standard 6: 500 µl of 0.1 µmol/mL standard + 500 µl chloroform; final concentration 0.05 µmol/mL. 4. Extraction Reagent (user-prepared): On the day before the experiment, prepare in an amber glass bottle by mixing n-heptane, anhydrous methanol, and chloroform at a volume ratio of 24:1:25. Mix thoroughly. Required User-Supplied Reagents: n-Heptane (C_7H_{16}, MW 100.20, CAS 142-82-5); anhydrous methanol (CH_4OH, MW 32.04, CAS 67-56-1); chloroform ($CHCl_3$, MW 119.38, CAS 67-66-3); absolute ethanol (C_2H_6O, MW 46.07, CAS 64-17-5).
Reaction conditions	Preheat Reagent 1 at 37°C for at least 30 min. After adding the test/control/standard/blank material, Extraction Reagent, and Reagent 1, shake for 10 min and centrifuge at 3,000 rpm for 10 min at room temperature. Transfer 200 µl of

	the upper layer, add 800 µ Reagent 2, shake for 2 min, stand at room temperature for 15 min, and measure absorbance at 550 nm within 30 min.

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