Free Aliphatic Acid Assay Kit (Microanalysis)

Description

Free aliphatic acids (Free Fatty Acids, FFA) are both products of lipid hydrolysis and substrates for lipid synthesis. The concentration of FFA is associated with lipid metabolism, glucose metabolism, and endocrine function, and can also reflect quality changes during food storage.

Detection Principle

FFA binds to copper ions to form fatty acid copper salts, which are soluble in chloroform. The copper ion content is determined using a copper reagent method, from which the FFA content can be calculated.

Packing

Taking 100T/96S packing for example:

Components	Packing	Storage
CB0080M-A	100 mL x 1	4 °C
CB0080M-B	10 mL x 1	Prepared one day prior to the experiment. In a glass bottle, mix n-heptane, anhydrous methanol, and chloroform at a ratio of 24:1:25 (v/v/v). Capped tightly, mixed thoroughly, and stored at room temperature.
CB0080M-C	4 mL x 1	4 °C
CB0080M-D	1 vial (powder) x 2	4 °C; Add 13 mL absolute ethanol before use, dissolve completely, stable at 4°C for 1 week
CB0080M-Standard	1 vial (powder) x 1	Stored at room temperature; Before use, transfer the reagent to a 10 mL glass vial, add 7.8 mL chloroform, and dissolve thoroughly to prepare a 5 μmol/mL palmitic acid standard solution.

Note: Perform pre-test with 2–3 samples before formal assay.

Instructions

I. Required Equipment & Materials:

Mortar, ice, centrifuge, pipettes, spectrophotometer/microplate reader, quartz cuvette/96-well plate, glass bottles, n-heptane, methanol, chloroform, ethanol, distilled water.

II. Sample Preparation:

1. Serum: Stored at -20°C.
2. Tissue: Rinse the tissue thoroughly with physiological saline, then remove surface moisture using absorbent paper. Weigh approximately 0.1 g, add 1.0 mL of CB0080M-A, and homogenize thoroughly. Centrifuge at 8000 rpm for 10 min at 4 °C, collect the supernatant.

III. Assay Procedure

1. Preheat the spectrophotometer or microplate reader for at least 30 min, set the wavelength to 550 nm, and zero the instrument with distilled water.
2. Preheat CB0080M-C at 37°C for at least 30mins.

3. Standard Preparation: Dilute the standard solution with chloroform to final concentrations of 1, 0.8, 0.6, 0.4, 0.2, 0.1, and 0.05 μmol/mL.

4. Sample measurement (add the following reagents sequentially into 1.5 mL centrifuge tubes):

	Control Tube (μL)	Assay Tube (μL)	Blank Tube (μL)	Standard Tube (μL)
Distilled Water	10			
Sample		10		
Chroloform			10	
Standard				10
CB0080M-B	100	100	100	100
CB0080M-C	40	40	40	40
Mix 10 min thoroughly, centrifuge(3000rpm), take 50 μL upper layer.				
Upper Phase	50	50	50	50
CB0080M-D	200	200	200	200
After vigorous mixing for 2 min, allow the mixture to stand for 15 min. Transfer 0.2 mL to a micro quartz cuvette or a 96-well plate, and measure the absorbance at 550 nm. Record the values as A_control, A_assay, A_blank, and A_standard, respectively.				

Note: Only one Control Tube and one Blank Tube are required.

IV. Calculation of FFA Content

1. Establishment of the Standard Curve:

Plot the standard curve using the concentration of the standard solutions as the x-axis and ΔA_standard (ΔA_standard = A_standard − A_blank) as the y-axis to obtain the regression equation y = kx + b. Substitute ΔA (ΔA = A_assay − A_control) into the equation to calculate x.

2. Calculation of FFA Content in Serum:

FFA (μmol/L) = 1000x

3. Calculation of FFA in Tissue:

(1) Based on protein concentration:

FFA (μmol/mg prot) = x × V_total ÷ (C_pr × V_total) = x ÷ C_pr

(2) Based on sample fresh weight:

FFA (μmol/g fresh weight) = x × V_total ÷ W

Note:

V_total: Total volume of supernatant, 1 mL

C_pr: Protein concentration of the sample, mg/mL

W: Fresh weight of the sample, g

1000: Unit conversion factor (1 L = 1000 mL)

Precautions

1. CB0080M-D should be prepared as late as possible. It is recommended to prepare it immediately before use, for example, when proceeding to the step of adding CB0080M-C.
2. Ensure that the mixing frequency and duration are consistent across all tubes.
3. The assay should be completed within 30 minutes. After measurement, all reaction mixtures should be properly sealed before disposal.
4. As most reagents are organic solvents, repeated pipetting with the same pipette tip may lead to volume inaccuracies; therefore, it is recommended to use a fresh tip for each transfer.
5. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
6. Please wear a lab coat and disposable gloves.