

Free Aliphatic Acid Assay Kit (Spectrophotometry)

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 50T/48S packing for example:

Component	Packing	Storage
CB0080S-A	50 mL x 1	4 °C
CB0080S-B	50 mL x 1 (self-prepared)	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.
CB0080S-C	10 mL x 1	Stored at room temperature
CB0080S-D	1 vial (powder) x 2	4 °C; Add 32 mL absolute ethanol before use, dissolve completely, stable at 4°C for 1 week
CB0080S-Standard	1 vial (powder) x 1	Stored at room temperature; Before use, add 7.8 mL chloroform, and dissolve thoroughly to prepare a 5µmol/ mL palmitic acid standard solution.

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

Instructions

I. Required Equipment & Materials:

Mortar, ice, benchtop centrifuge, visible spectrophotometer, 1 mL glass cuvette, adjustable pipettes, a 50 mL glass bottle, a 10 mL glass bottle, n-heptane, anhydrous methanol, chloroform (trichloromethane), absolute ethanol, and 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 CB0080S-A, and homogenize thoroughly. Centrifuge at 8000 rpm for 10 min at 4 °C, collect the supernatant.

III. Assay Procedure

1. Preheat the spectrophotometer for at least 30 min, set the wavelength to 550 nm, and zero the instrument with distilled water.
2. Preheat CB0080S-C at 37°C for at least 30mins.

- 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 $\mu\text{mol/mL}$.
- 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	50			
Sample		50		
Chloroform			50	
Standard				50
CB0080S-B	500	500	500	500
CB0080S-C	200	200	200	200
Mix 10 min thoroughly, centrifuge(3000rpm), take 200 μL upper layer.				
Upper Phase	200	200	200	200
CB0080S-D	800	800	800	800
After vigorous mixing for 2 min, allow the mixture to stand for 15 min. 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

- Establishment of the Standard Curve:

Plot the standard curve using the concentration of the standard solutions as the x-axis and $\Delta A_{\text{standard}}$ ($\Delta A_{\text{standard}} = A_{\text{standard}} - A_{\text{blank}}$) as the y-axis to obtain the regression equation $y = kx + b$. Substitute ΔA ($\Delta A = A_{\text{assay}} - A_{\text{control}}$) into the equation to calculate x.

- Calculation of FFA Content in Serum:

$$\text{FFA } (\mu\text{mol/L}) = 1000x$$

- Calculation of FFA in Tissue:

- (1) Based on protein concentration:

$$\text{FFA } (\mu\text{mol/mg prot}) = x \times V_{\text{total}} \div (C_{\text{pr}} \times V_{\text{total}}) = x \div C_{\text{pr}}$$

- (2) Based on sample fresh weight:

$$\text{FFA } (\mu\text{mol/g fresh weight}) = x \times V_{\text{total}} \div W$$

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. CB0080S-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 CB0080S-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.

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