CREATIVE BIOMART®
Assay Kit

Free fatty acid determination kit

Product Information

Cat.No. Kit-2164

Product Overview

FFA is both a product of fat hydrolysis and a substrate for fat synthesis. The concentration of FFA in serum is related to lipid metabolism, glucose metabolism and endocrine function. FFA combines with copper ions to form fatty acid copper salts, which are dissolved in chloroform; the content of free fatty acids can be calculated by measuring the content of copper ions by the copper reagent method.

Size

50T/48S

Kit Components

Extraction solution: 50 mL×1 bottle, store at 2-8°C
Reagent 1: 50 mL × 1 bottle (self-provided), stored at room temperature, one day before the experiment, take a glass bottle and prepare it according to the ratio of n-heptane: anhydrous methanol: chloroform = 24:1:25 (self-provided), cover Mix immediately.
Reagent 2: 16 mL×1 bottle, stored at room temperature
Reagent 3: powder × 2 bottles, store at 2-8°C, add 32 mL of absolute ethanol to each bottle before use to fully dissolve, and store at 2-8°C for one week.
Standard product: powder × 1, stored at room temperature, 10mg palmitic acid. Before use, transfer the reagent to a 10 mL glass bottle and add 7.8 mL of chloroform to dissolve it fully, which is a 5 μmol/mL palmitic acid standard solution.

Materials Required but Not Supplied

Mortar/homogenizer, ice, benchtop centrifuge, visible spectrophotometer, 1mL glass cuvette, adjustable pipette, one 40mL glass bottle, one 10mL glass bottle, n-heptane, anhydrous methanol, chloroform (chloroform), absolute ethanol and distilled water.

Technical Notes

1. Reagent 3 should be prepared as late as possible. Reagent 3 can be prepared when reagent 2 is

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added.
2. It is necessary to ensure that the oscillation frequency and time of each tube are consistent.
3. Try to complete the measurement within 30 minutes, and seal it before discarding after the measurement.
4. Since most of the reagents used are organic solvents, multiple suctions with the same tip will cause the volume to be inaccurate. It is recommended to replace the tip.
5. Detection limit: 0.012 μmol/L
6. Linear range: 0.025-0.8 μmol/L

Preparation

1. Serum sample: After standing for 1 h at room temperature, the blood was centrifuged at 3500 rpm for 15 min at 4 °C, and the upper serum was taken and stored at 2-8 °C until testing.
2. Tissue sample: After the tissue is washed with normal saline, absorb the surface water with absorbent paper, weigh about 0.1 g, add 1.0 mL of extract, and after homogenization, centrifuge at 8000 rpm and 4 ° C for 10 min, take the supernatant, wait for Measurement.

Assay Protocol

Note: Before the formal measurement, be sure to take 2-3 samples with large expected differences for pre-determination.
1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 550 nm, and zero in anhydrous ethanol.
2. Preheat reagent 2 in a 37°C water bath for more than 30 minutes.
3. Dilution of the standard: Dilute the standard with chloroform to 0.8, 0.6, 0.4, 0.2, 0.1, 0.05, 0.025 μmol/mL.
4. Add the corresponding reagents to the 1.5mL centrifuge tube according to the table below
Reagent name (μL) Assay tube Control tube Standard tube Blank tube
Sample 50 - - -
Distilled water - 50 - -
Standard - - 50 -
Chloroform - - - 50
Reagent one 500

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Reagent II 200
After fully shaking for 10min, centrifuge at 3000rpm for 10min
Top solution 200
Reagent Three 800
After fully oscillating for 2 min, let stand for 15 min, and measure the absorbance at 550 nm, which are recorded as A measuring tube, A control tube, A standard tube, and A blank tube.
(Make only one control tube and one blank tube each)

Analysis

1. Drawing of the standard curve:
With the concentration of the standard solution as the x-axis and the ΔA standard (ΔA=A standard tube - A blank tube) as the y-axis, draw a standard curve to obtain the equation y=kx+b. Put ΔA (ΔA=A
Assay tube - A control tube) into the equation to get x.
2. Calculation of FFA content in serum
FFA (μmol/L)=1000x
3. Calculation of FFA content in tissues
(1) Calculated according to the sample protein concentration
FFA content (μmol/mg prot)=x×V sample total ÷ (Cpr×V sample total)=x÷Cpr
(2) Calculated by sample quality
FFA (μmol/g mass) = x×V sample total ÷ W
V sample total: total volume of supernatant, 1 mL; Cpr: sample protein concentration, mg/mL; W: sample mass, g. 1000: unit conversion factor, 1L=1000mL.

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