Lipoprotein lipase Assay Kit (Microanalysis)

Description

Lipoprotein lipase (LPL) is an enzyme produced by adipocytes, cardiomyocytes, skeletal muscle cells, mammary cells, macrophages, and other parenchymal cells. It hydrolyzes triglycerides into free fatty acids and monoacylglycerols, providing substrates for energy metabolism or lipid storage. The physiological roles of LPL vary across different tissues, reflecting its tissue-specific functions in lipid metabolism.

Detection Principle

Lipoprotein lipase hydrolyzes p-nitrophenyl palmitate to generate p-nitrophenol, which exhibits a characteristic absorption peak at 400 nm.

Packing

Taking 100T/48S packing for example:

Components	Packing	Storage
CB0093M-A	80 mL x 1	4 °C
CB0093M-B	1 mL x 1	Store at 4°C, protected from light. Before use, dissolve in 1 mL of acetone.
CB0093M-C	20 mL x 1	4 °C
CB0093M-Standard（5 μmol/mL）	1 mL x 1	4 °C
Before the formal assay, it is recommended to perform a preliminary test using 2–3 samples expected to show relatively large differences.		

Instructions

I. Preparation of Lab Instruments

Analytical balance, refrigerated centrifuge, mortar and pestle, water bath, visible spectrophotometer or microplate reader, micro-volume glass cuvettes or 96-well microplates, ice, acetone, and distilled water.

II. Crude Enzyme Extraction

1. For Tissues:

Add CB0093M-A at a ratio of tissue weight (g) : CB0093M-A volume (mL) = 1:5–10 (recommended: weigh approximately 0.1 g of tissue and add 1 mL of CB0093M-A). Homogenize the tissue in an ice bath, then centrifuge at 10,000 × g for 10 min at 4°C. Collect the supernatant and keep it on ice for subsequent analysis.

2. For Cells:

Add CB0093M-A at a ratio of cell number (×10⁴ cells) : CB0093M-A volume (mL) = 500–1000:1 (recommended: add 1 mL of CB0093M-A to 5 × 10⁶ cells). Disrupt the cells by sonication in an ice bath (300 W power, 3 s on / 7 s off, total sonication time: 3 min). Centrifuge at 10,000 × g for 10 min at 4°C, then collect the supernatant for analysis.

3. For Serums:

Use the serum sample directly for the assay without extraction.

III. Assay Procedure

1. Preheat the spectrophotometer for 30 minutes, set the wavelength to 400 nm, and zero the instrument using distilled water.
2. Dilute the 5 μmol/mL standard solution 16-fold with CB0093M-A to prepare a 0.3125 μmol/mL standard solution for use.

3. Add the following reagents sequentially into a 1.5 mL centrifuge tube:

	Control Tube（μL）	Sample Tube（μL）	Standard Tube（μL）	Blank Tube（μL）
Crude Enzyme	30	30		
Standard Solution			30	
Distilled Water				30
CB0093M-A	120	108	120	120
CB0093M-B		12		
Mix well and incubate in a 45°C water bath for 10 min.				
CB0093M-C	150	150	150	150
After thorough mixing, allow to stand for 2 min. Then centrifuge the control tube and the assay tube at 8000 g for 10 min at room temperature. Transfer 200 μL of the supernatant from the control tube and the assay tube, as well as the standard tube and blank tube, into a micro glass cuvette or a 96-well plate. Measure the absorbance at 400 nm and record it as A_control, A_sample, A_blank, and A_standard. Calculate: ΔA = A_sample − A_control ΔA_standard = A_standard − A_blank				

IV. Calculation of LPL Activity

1. Calculation of Serum (Plasma) LPL Activity

Unit Definition:

One unit of LPL activity is defined as the amount of enzyme that hydrolyzes 1 nmol of 4-nitrophenol per minute per milliliter of serum at 45°C and pH 7.5.

Calculation Formula:

LPL (U/mL) = ΔA ÷ (ΔA_standard ÷ C_standard) ÷ T × 1000 = 31.25 × ΔA ÷ ΔA_standard

2. Calculation of LPL Activity in Tissue, Bacteria, or Cells

(1) Based on Tissue Weight

Unit Definition:

One unit of LPL activity is defined as the amount of enzyme that hydrolyzes 1 nmol of 4-nitrophenol per minute per gram of tissue at 45°C and pH 7.5.

Calculation Formula:

LPL (U/g tissue) = ΔA ÷ (ΔA_standard ÷ C_standard) × V_extract ÷ W ÷ T × 1000 = 31.25 × ΔA ÷ ΔA_standard÷ W

(2) Based on Protein Concentration

Unit Definition:

One unit of LPL activity is defined as the amount of enzyme that hydrolyzes 1 nmol of 4-nitrophenol per minute per milligram of protein at 45°C and pH 7.5.

Calculation Formula:

LPL (U/mg protein) = ΔA ÷ (ΔA_standard ÷ C_standard) × V_extract ÷ (V_extract × Cpr) ÷ T × 1000 = 31.25 × ΔA ÷ ΔA_standard ÷ Cpr

(3) Based on Cell or Bacterial Number

Unit Definition:

One unit of LPL activity is defined as the amount of enzyme that hydrolyzes 1 nmol of 4-nitrophenol per minute per 10^4 cells at 45°C and pH 7.5.

Calculation Formula:

LPL (U/10^4 cells) = ΔA ÷ (ΔA_standard ÷ C_standard) × V_extract ÷ 500 ÷ T × 1000 = 0.0625 × ΔA ÷ ΔA_standard

Note:

C_standard: Concentration of the standard solution, 0.3125 μmol/mL

V_extract: Volume of CB0093M-A extraction buffer added, 1 mL

T: Reaction time, 10 min

Cpr: Protein concentration of the sample (mg/mL)

W: Tissue weight (g)

500: Total number of bacteria or cells, 5 × 10^6

1000: Unit conversion factor (1 μmol = 1000 nmol)

Precautions

1. After adding CB0093M-C, mix thoroughly and let the reaction stand for 2 minutes before measuring immediately. Delayed measurement may affect the absorbance value.
2. It is normal for the reaction mixture to become turbid after adding CB0093M-B to the assay tube.
3. If the absorbance is too high or the assay results are unstable, appropriately dilute the crude enzyme solution and multiply the calculated result by the corresponding dilution factor.
4. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
5. Please wear a lab coat and disposable gloves.