Lipoprotein Lipase (LPL) Activity Assay Kit (ELISA)

Cat. No. KITG-057 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	The Lipoprotein Lipase (LPL) Activity Assay Kit is designed for the quantitative determination of lipoprotein lipase (LPL) activity in tissue, bacteria, cells, serum (plasma), and culture medium samples. Lipoprotein lipase (LPL) is mainly synthesized and secreted in parenchymal cells of organs. It can catalyze the hydrolysis of triglycerides to fatty acids and monoacylglycerol. As the rate-limiting enzyme for triglyceride degradation, it plays an important role in lipid absorption, transport, clearance, and metabolism. The assay is based on the enzymatic reaction where LPL catalyzes the hydrolysis of 4-nitrophenyl palmitate to generate 4-nitrophenol. The product has a characteristic absorption peak at 400 nm, and the LPL activity is characterized by the change in absorbance value.
Species	Human, Animal, Bacteria, Cell
Source	ELISA
Description	Principle: Lipoprotein lipase (LPL) is mainly synthesized and secreted in parenchymal cells of organs. It can catalyze the hydrolysis of triglycerides to fatty acids and monoa cylglycerol. As the rate-limiting enzyme for triglyceride degradation, it plays an import ant role in lipid absorption, transport, clearance, and metabolism. Lipoprotein lipase c an catalyze the hydrolysis of 4-nitrophenyl palmitate to generate 4-nitrophenol. The pr oduct has a characteristic absorption peak at 400 nm, and the LPL activity is characte rized by the change in absorbance value. Intended Use: For research use only. Not fo r diagnostic or clinical use. Sample Types: Tissue, bacteria, cells, serum (plasma), an d culture medium. Detection Method: Microanalysis Methods. Detection Wavelength:

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400 nm. Reaction Conditions: 45°C, pH = 7.5, 10 min. Specification: 100T/48S (100 assays/48 samples). Storage Temperature: 2-8°C. Kit Components: - Reagent 1: Liquid, 80 mL × 1 bottle, store at 4°C - Reagent 2: Powder × 1 vial, store at 4°C. Before use, add 1.5 mL acetone to fully dissolve - Reagent 3: Liquid, 20 mL × 1 bottle, store at 4°C - Standard Solution: Liquid, 1 mL × 1 vial, store at 4°C. 5 μmol/mL 4-nitrophenol standard solution Standard Application Solution Preparation: Before use, dilute the 5 μmol/mL 4-nitrophenol standard solution with Reagent 1 by 16-fold to 0.3125 μmol/mL as the standard application solution. Note: The reagents must be stored strictly according to the preservation conditions. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents. Materials Required but Not Provided: - Visible spectrophotometer or microplate reader - Micro glass cuvette (path length 10 mm) or 96-well plate - Mortar/homogenizer - Adjustable pipette - Benchtop centrifuge - Constant temperature water bath/incubator - Acetone (CH_3COCH_3, MW = 58.08, CAS: 67-64-1) - Distilled water Sample Preparation: 1. Bacteria or Cells: Centrifuge to collect bacteria or cells into centrifuge tubes. Process samples according to the ratio of bacteria or cell number (10^4 cells) : Reagent 1 volume (mL) = (500-1000) : 1 (recommended: 5 million bacteria or cells add 1 mL Reagent 1), sonicate on ice (power 20% or 200 W, ultrasound 3 s, interval 10 s, repeat 30 times), centrifuge at 4°C 10 000 × g for 10 min, take the supernatant and place on ice for detection. 2. Tissue: Process samples according to the ratio of tissue mass (g) : Reagent 1 volume (mL) = 1 : (5-10) (recommended: weigh 0.1 g tissue, add 1 mL Reagent 1), homogenize on ice, centrifuge at 4°C 10000 × g for 10 min, take the supernatant and place on ice for detection. 3. Serum (Plasma), Culture Medium and Other Liquid Samples: Detect directly or dilute appropriately before detection. Assay Procedure: 1. Preheat the spectrophotometer or microplate reader for more than 30 min, adjust the wavelength to 400 nm, and zero with distilled water. 2. Add the following reagents sequentially in centrifuge tubes: Test Tube (μL): - Crude enzyme solution: 30 μL - Reagent 1: 108 μL - Reagent 2: 12 μL Mix thoroughly, react accurately at 45°C f

or 10 min - Reagent 3: 150 μL Mix thoroughly, let stand at room temperature for 2 min Centrifuge at 8000 × g at room temperature for 10 min, take the supernatant Control Tube (μL): - Crude enzyme solution: 30 - Reagent 1: 120 μL Mix thoroughly, react ac curately at 45°C for 10 min - Reagent 3: 150 μL Mix thoroughly, let stand at room tem perature for 2 min Centrifuge at 8000 × g at room temperature for 10 min, take the su pernatant Standard Tube (μL): - Standard application solution: 30 μL - Reagent 1: 12 0 μL μL Mix thoroughly, react accurately at 45°C for 10 min - Reagent 3: 150 μL Mix t horoughly, let stand at room temperature for 2 min Centrifuge at 8000 × g at room te mperature for 10 min, take the supernatant Blank Tube (μL): - Distilled water: 30 μL - Reagent 1: 120 μL μL Mix thoroughly, react accurately at 45°C for 10 min - Reagent 3: 150 μL Mix thoroughly, let stand at room temperature for 2 min Centrifuge at 8000 × g at room temperature for 10 min, take the supernatant Absorbance Measurement: Pipette 200 μL of the supernatant into a micro glass cuvette or 96-well plate, measure the absorbance at 400 nm, record as A_test, A_control, A_standard and A_blank. Cal culate ΔA_test = A_test - A_control, ΔA_standard = A_standard - A_blank. Note: The blank tube only needs to be measured 1-2 times. Each sample needs to set up a cont rol tube. Calculation: 1. Calculated by tissue sample mass: Unit Definition: Under 45° C pH = 7.5 conditions, one enzyme activity unit is defined as the amount of enzyme th at generates 1 nmol of 4-nitrophenol per g of tissue per minute. LPL (U/g) = (C_stand ard × ΔA_test × V_extract × V_total × 1000) / (ΔA_standard × W × T) = (31.25 × ΔA_t est) / (ΔA_standard × W) 2. Calculated by tissue protein concentration: Unit Definition: Under 45°C pH = 7.5 conditions, one enzyme activity unit is defined as the amount of enzyme that generates 1 nmol of 4-nitrophenol per mg of tissue protein per minute. L PL (U/mg prot) = (C_standard × ΔA_test × V_extract × 1000) / (ΔA_standard × Cpr × T) = (31.25 × ΔA_test) / (ΔA_standard × Cpr) 3. Calculated by bacteria or cell number: Unit Definition: Under 45°C pH = 7.5 conditions, one enzyme activity unit is defined as the amount of enzyme that generates 1 nmol of 4-nitrophenol per 10^4 bacteria or cell s per minute. LPL (U/10^4 cell) = (C_standard × ΔA_test × V_extract × V_total × 1000) / (ΔA_standard × Cell number × T) = (31.25 × ΔA_test) / (ΔA_standard × Cell number)

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	4. Calculated by liquid sample volume: Unit Definition: Under 45°C pH = 7.5 conditions, one enzyme activity unit is defined as the amount of enzyme that generates 1 nmol of 4-nitrophenol per mL of liquid sample per minute. LPL (U/mL) = (C_standard × ΔA_test × 1000) / (ΔA_standard × T) = (31.25 × ΔA_test) / ΔA_standard Notes: C_standard: Standard application solution concentration, 0.3125 μmol/mL. V_extract: Total volume of crude enzyme solution, 1 mL. Cpr: Protein concentration of sample, mg/mL. W: Sample mass, g. Cell number: In units of 10^4. T: Reaction time, 10 min. 1000: Unit conversion coefficient, 1 μmol = 1000 nmol. Cautions: 1. It is normal for the test tube to become turbid after adding Reagent 2. 2. If A_test is greater than 1.5, it is recommended to dilute the crude enzyme solution with Reagent 1 appropriately before measurement, and modify accordingly during calculation. 3. To ensure accurate results and avoid reagent loss, please read the instructions carefully before measurement (subject to the actual received instructions), confirm whether reagent storage and preparation are sufficient, and whether the operating steps are clear. Be sure to take 2-3 samples with expected large differences for pre-determination, and contact staff in time if you have any questions during the process.
Form	Activity Assay Kit (ELISA, 100T/48S)
Applications	Quantitative determination of lipoprotein lipase (LPL) activity in tissue, bacteria, cells, serum (plasma), and culture medium samples. Suitable for research use in studies related to lipid metabolism, triglyceride degradation, lipid absorption, transport, and clearance.
Notes	1. It is normal for the test tube to become turbid after adding Reagent 2. 2. If A_test is greater than 1.5, it is recommended to dilute the crude enzyme solution with Reagent 1 appropriately before measurement, and modify accordingly during calculation. 3. To ensure accurate results and avoid reagent loss, please read the instructions carefully before measurement (subject to the actual received instructions), confirm whether reagent storage and preparation are sufficient, and whether the operating steps are

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	clear. Be sure to take 2-3 samples with expected large differences for pre-determination, and contact staff in time if you have any questions during the process.
Storage	2-8°C. Reagent 1, Reagent 2, Reagent 3, and Standard solution store at 4°C.
Concentration	Standard concentration: 5 μmol/mL 4-nitrophenol. Standard application solution: 0.3125 μmol/mL (diluted 16-fold with Reagent 1).
Shipping	Shipped at ambient temperature. Upon receipt, store at 2-8°C according to component requirements.
Reconstitution	Reagent 2: Add 1.5 mL acetone to fully dissolve before use. Standard application solution: Dilute 5 μmol/mL 4-nitrophenol standard solution with Reagent 1 by 16-fold to 0.3125 μmol/mL.
Usage	For research use only. Not for diagnostic or clinical use.

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