Lipase (LPS) Activity Assay Kit

Cat. No. KITG-054 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	The Lipase (LPS) Activity Assay Kit is designed for the quantitative determination of lipase (LPS) activity in tissue, bacteria, cells, serum, and culture medium samples. Lipase (LPS), also known as glycerol ester hydrolase, belongs to the enzyme class that catalyzes the hydrolysis and formation of long-chain ester bonds. It has affinity for the oil-water interface and can catalyze the hydrolysis of triglycerides to generate fatty acids and glycerol (or diglycerides and monoesters). Lipase is widely present in animal and plant tissues and microorganisms, and its activity determination is of great significance for fat metabolism analysis. The assay is based on the copper soap method: lipase catalyzes the hydrolysis of oil esters into free fatty acids, which react with copper ions in the chromogenic reagent to form copper soap blue complexes. The product has a characteristic absorption peak at 710 nm, and the lipase activity is characterized by the change in absorbance.
Species	Human, Animal, Plant, Microorganism
Source	Visible Spectrophotometry
Description	Principle: Lipase (LPS), also known as glycerol ester hydrolase, belongs to the enzyme class that catalyzes the hydrolysis and formation of long-chain ester bonds. It has affinity for the oil-water interface and can catalyze the hydrolysis of triglycerides to generate fatty acids and glycerol (or diglycerides and monoesters). Lipase is widely present in animal and plant tissues and microorganisms, and its activity determination is of great significance for fat metabolism analysis. Lipase catalyzes the hydrolysis of oil esters into free fatty acids. The fatty acid generation rate is determined by the

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copper soap method: fatty acids react with copper ions in the chromogenic reagent to form copper soap blue complexes. The product has a characteristic absorption peak at 710 nm, and the lipase activity is characterized by the change in absorbance value. Intended Use: For research use only. Not for diagnostic or clinical use. Sample Types: Tissue, bacteria, cells, serum, and culture medium. Detection Method: Visible Spectrophotometry. Detection Wavelength: 710 nm. Specification: 70T/50S (70 assays/50 samples). Storage Temperature: 2-8°C. Kit Components: - Reagent 1: Liquid, 80 mL × 1 bottle, store at 4°C - Reagent 2: Liquid, 10 mL × 1 bottle, store at room temperature protected from light - Reagent 3: Liquid, 20 mL × 1 bottle, store at 4°C - Standard Solution: Liquid × 1 vial, store at 4°C protected from light. Before use, add 1 mL anhydrous ethanol to fully dissolve (this is 120 μmol/mL oleic acid standard solution) Standard Dilution Preparation: Before use, dilute the 120 μmol/mL oleic acid standard solution with anhydrous ethanol to 60, 30, 15, 7.5, 3.75, 1.875 μmol/mL as standard dilutions. Standard Dilution Table: Tube 1: Dilute 120 μmol/mL standard 300 μL + anhydrous ethanol 300 μL → 60 μmol/mL Tube 2: Dilute 60 μmol/mL standard 300 μL + anhydrous ethanol 300 μL → 30 μmol/mL Tube 3: Dilute 30 μmol/mL standard 300 μL + anhydrous ethanol 300 μL → 15 μmol/mL Tube 4: Dilute 15 μmol/mL standard 300 μL + anhydrous ethanol 300 μL → 7.5 μmol/mL Tube 5: Dilute 7.5 μmol/mL standard 300 μL + anhydrous ethanol 300 μL → 3.75 μmol/mL Tube 6: Dilute 3.75 μmol/mL standard 300 μL + anhydrous ethanol 300 μL → 1.875 μmol/mL 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 - 1 mL glass cuvette (path length 10 mm) - Mortar/homogenizer - Adjustable pipette - Benchtop centrifuge - Constant temperature water bath/incubator - Anhydrous ethanol (C_2H_6O, MW = 46.07, CAS: 64-17-5) - Toluene (C_7H_8, MW = 92.14, CAS: 108-88-3) - Distilled water Sample Preparation: 1. Tissue: Process samples according to the ratio of tissue mass (g) : Reagent 1 volume (mL) = 1 : (5-10)

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(recommended: weigh 0.1 g tissue, add 1 mL Reagent 1), homogenize on ice, centrifuge at 4°C 12000 × g for 10 min, take the supernatant and place on ice for detection. 2. 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 300 W, ultrasound 3 s, interval 7 s, total time 3 min), centrifuge at 4°C 12000 × g for 10 min, take the supernatant and place on ice for detection. 3. Serum, Culture Medium and Other Liquid Samples: Detect directly or dilute appropriately with Reagent 1 before detection. Assay Procedure: 1. Preheat the spectrophotometer for more than 30 min, adjust the wavelength to 710 nm, and zero with toluene. 2. Before the test, preheat Reagent 1 and Reagent 2 at 37°C for 30 min. 3. Add the following reagents sequentially in 2 mL centrifuge tubes: Standard Tube (μL): - Reagent 1: 375 - Reagent 2: 125 Mix thoroughly by repeated shaking - Standard dilution: 200 - Mix thoroughly by shaking - Incubate at 37°C for exactly 10 min - Toluene: 1000 Mix thoroughly by repeated shaking, centrifuge at 8000 × g at room temperature for 10 min, take the supernatant - Supernatant: 900 - Reagent 3: 225 Mix thoroughly by repeated shaking, centrifuge at 8000 × g at room temperature for 10 min Blank Tube (μL): - Reagent 1: 375 - Reagent 2: 125 Mix thoroughly by repeated shaking - Distilled water: 200 - Mix thoroughly by shaking - Incubate at 37°C for exactly 10 min - Toluene: 1000 Mix thoroughly by repeated shaking, centrifuge at 8000 × g at room temperature for 10 min, take the supernatant - Supernatant: 900 - Reagent 3: 225 Mix thoroughly by repeated shaking, centrifuge at 8000 × g at room temperature for 10 min Test Tube (μL): - Reagent 1: 375 - Reagent 2: 125 Mix thoroughly by repeated shaking - Crude enzyme solution: 200 - Mix thoroughly by shaking - Incubate at 37°C for exactly 10 min - Toluene: 1000 Mix thoroughly by repeated shaking, centrifuge at 8000 × g at room temperature for 10 min, take the supernatant - Supernatant: 900 - Reagent 3: 225 Mix thoroughly by repeated shaking, centrifuge at 8000 × g at room temperature for 10 min Absorbance Measurement:

Pipette 800 μL of the upper layer solution into a 1 mL glass cuvette, measure the absorbance at 710 nm, record as A_test, A_standard and A_blank. Calculate ΔA_test = A_test - A_blank, ΔA_standard = A_standard - A_blank. Note: The blank tube only needs to be measured 1-2 times. Standard Curve Establishment: Use 60, 30, 15, 7.5, 3.75, 1.875 μmol/mL as the x-axis, and the corresponding ΔA_standard as the y-axis to plot the standard curve, obtain the standard equation y = kx + b, and substitute ΔA_test into the formula to obtain x (μmol/mL). Calculation: 1. Calculated by tissue protein concentration: Unit Definition: Under 37°C conditions, one enzyme activity unit is defined as the amount of enzyme that generates 1 μmol of fatty acid per mg of tissue protein per minute. LPS (U/mg prot) = (x × V_sample) / (Cpr × T) = (0.1 × x) / Cpr 2. Calculated by tissue sample mass: Unit Definition: Under 37°C conditions, one enzyme activity unit is defined as the amount of enzyme that generates 1 μmol of fatty acid per g of tissue per minute. LPS (U/g) = (x × V_sample × V_extract) / (W × T) = (0.1 × x) / W 3. Calculated by bacteria or cell number: Unit Definition: Under 37°C conditions, one enzyme activity unit is defined as the amount of enzyme that generates 1 μmol of fatty acid per 10^4 bacteria or cells per minute. LPS (U/10^4 cell) = (x × V_sample × V_extract) / (Cell number × T) = (0.1 × x) / Cell number 4. Calculated by liquid sample volume: Unit Definition: Under 37°C conditions, one enzyme activity unit is defined as the amount of enzyme that generates 1 μmol of fatty acid per mL of liquid sample per minute. LPS (U/mL) = (x × V_sample) / T = 0.1 × x Notes: V_sample: Volume of crude enzyme solution added to the reaction system, 0.2 mL. V_extract: Total volume of crude enzyme solution, 1 mL. Cpr: Protein concentration of crude enzyme solution, mg/mL. W: Sample mass, g. Cell number: In units of 10^4. T: Reaction time, 10 min. Cautions: 1. Keep away from fire sources during the experiment. Toluene is toxic; it is recommended to operate in a fume hood and take protective measures. 2. If the measured absorbance exceeds the linear range of the standard absorbance: If higher than the maximum value, it is recommended to dilute the crude enzyme solution with Reagent 1 appropriately before measurement; if lower than the minimum value, it is recommended to

	increase the sample amount 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 (Visible Spectrophotometry, 70T/50S)
Applications	Quantitative determination of lipase (LPS) activity in tissue, bacteria, cells, serum, and culture medium samples. Suitable for research use in studies related to fat metabolism, lipid metabolism, and triglyceride hydrolysis.
Notes	1. Keep away from fire sources during the experiment. Toluene is toxic; it is recommended to operate in a fume hood and take protective measures. 2. If the measured absorbance exceeds the linear range of the standard absorbance: If higher than the maximum value, it is recommended to dilute the crude enzyme solution with Reagent 1 appropriately before measurement; if lower than the minimum value, it is recommended to increase the sample amount 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.
Storage	2-8°C. Reagent 1 and Reagent 3 store at 4°C; Reagent 2 store at room temperature protected from light; Standard solution store at 4°C protected from light.

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Concentration	Standard concentration: 120 μmol/mL (after dissolving with 1 mL anhydrous ethanol). Standard dilutions: 60, 30, 15, 7.5, 3.75, 1.875 μmol/mL.
Shipping	Shipped at ambient temperature. Upon receipt, store at 2-8°C according to component requirements.
Reconstitution	Standard Solution: Add 1 mL anhydrous ethanol to fully dissolve before use (120 μmol/mL oleic acid standard). Standard dilutions: Dilute with anhydrous ethanol to 60, 30, 15, 7.5, 3.75, 1.875 μmol/mL.
Usage	For research use only. Not for diagnostic or clinical use.

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