(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Total Lipase Assay Kit

Catalog No.: BC00196

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Total Lipase Assay Kit
Detection Method	Colorimetric
Sample Type	Serum, plasma, tissue
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (550 nm)

Product Introduction

Lipoprotein lipase (LPL) is located on the surface of capillary endothelial cells in extrahepatic tissues. It mainly catalyzes the hydrolysis of triglycerides in plasma chylomicrons (CM) and very low-density lipoproteins (VLDL), and plays an important role in the degradation of CM and VLDL.

Hepatic lipase (HL) is present on the surface of hepatic endothelial cells and mainly participates in the metabolism of intermediate-density lipoproteins (IDL) and high-density lipoproteins (HDL).

Principle

Lipoprotein lipase (LPL) and hepatic lipase (HL) can decompose triglycerides (TG) and hydrolyze them into glycerol and free fatty acids (FFA). The activities of lipoprotein lipase (LPL) and hepatic lipase (HL) can be calculated by determining the amount of produced free fatty acids (FFA) with copper reagent.

Hepatic lipase is a glycoprotein. Its activity does not require activation by apolipoprotein C II or certain ions, nor is it inhibited by high-concentration salts or protamine. Based on this characteristic, the activities of lipoprotein lipase (LPL) and hepatic lipase (HL) can be determined separately.

Components

No.	Components	Size	Notes
Reagent 1	Liquid	5 mL×1	Preparation of Reagent 1: Add 1.5 mL of solvent to each powder vial to dissolve completely before use, and store at 4°C.
	Powder	2 vials	
Reagent 2	Promoter	6 mL×1	Store at 4°C.
	Inhibitor	6 mL×1	Store at 4°C.
Reagent 3	Liquid	6 mL×1	Store at 4°C.
Reagent 4	Solution A	0.5 mL×1	Store at 4°C. Preparation of Reagent 4: Mix Solution A and Solution B at a ratio of 1:200. Prepare freshly before use. After preparation, pre-incubate in a 37°C water bath for no less than 10 minutes.
	Solution B	30 mL×1	
Reagent 5	Solution A	4 mL×1	Preparation of Copper Reagent: Prepare the reagent by mixing Solution A: Solution B: Solution C at a ratio of 2:1:17. The prepared copper reagent should be a clear liquid. Prepare it fresh for immediate use. Do not mix leftover reagent with newly prepared copper reagent, otherwise the assay results will be affected. Pre-warm the prepared reagent in a 37°C water bath for no less than 10 minutes before use. Prepare the copper reagent strictly in the order of Solution A, Solution B and Solution C, and mix well after adding each reagent.
	Solution B	4 mL×1	
	Solution C	30 mL×1	
Reagent 6	Powder A	2 vials	Preparation of Chromogenic Reagent: Dissolve one vial of Powder A completely with one vial of 10 mL Solution B before use. The mixture can be stored at -20°C. Add Solution C at a ratio of 100:1 within 1-2 minutes prior to use, mix thoroughly and use
	Solution B	10 mL×2	

	Solution C	0.5 mL×1	immediately. Prepare fresh for immediate use only; any unused chromogenic reagent shall not be reused.
Reagent 7	Standard powder	3 vials	Store at 4°C.
	Solvent	60 mL×1	

Preparation of 2 mmol/L Palmitic Acid Standard Stock Solution: Take one vial of standard powder, dissolve it with the solvent and make up the volume to 10 mL. Mix thoroughly completely.

Preparation of 500 μmol/L Palmitic Acid Standard: Dilute the 2 mmol/L palmitic acid standard stock solution 4-fold with solvent, namely dilute at the ratio of 2 mmol/L palmitic acid standard stock solution: solvent = 1:3.

Storage

The validity period of the kit is 3 months.

Preparation

Spectrophotometer with a wavelength of 550 nm and 1 cm optical path cuvettes, 37°C water bath or constant temperature incubator, chloroform, desktop low-speed centrifuge, pipettors of various specifications, double-distilled water, chloroform (analytical grade), 0.9% normal saline or 0.1M PBS, vortex mixer, glass test tubes or centrifuge tubes, epidural anesthesia puncture needle core and sleeve together with matching glass syringes, protein assay reagents (for tissue samples, available from our company).

- **Sample handling**

1. Serum (plasma) samples: use directly.
2. Tissue samples: Weigh the tissue sample. Add 9 times the volume of normal saline at a mass-to-volume ratio of 1 g: 9 mL. Perform mechanical homogenization in an ice water bath to prepare 10% tissue homogenate. Centrifuge at 2500 rpm for 10 minutes, and collect the supernatant for subsequent determination.

Operation process

It is recommended to use a glass syringe together with the needle core and sleeve of an epidural anesthesia puncture needle when aspirating the lower extract.

	Blank tube	Standard tube	LPL Assay tube	HL Assay tube
Double-distilled water (mL)	0.3	0.25	0.1	
500 μmol/L Palmitic Acid Standard (mL)		0.05		
Sample (mL)			0.05	0.05
Reagent 1 (mL)			0.05	0.05
Mix thoroughly				
Reagent 2 Promoter (mL)				0.1
Reagent 2 Inhibitor (mL)			0.1	
Reagent 3 (mL)				0.1
Mix thoroughly				
Reagent 4 (mL), Pre-warm at 37°C	0.5	0.5	0.5	0.5
Vortex and mix thoroughly, then incubate in a 37°C water bath for 20 minutes.				
Copper Reagent (mL), Pre-warm at 37°C	0.5	0.5	0.5	0.5
Vortex for 10 seconds, then incubate in a 37°C water bath for 10 minutes.				
Chloroform (mL), Pre-warm at 37°C	3.0	3.0	3.0	3.0
Incubate in a 37°C water bath for 10 minutes, then thoroughly mix with a vortex mixer and perform extraction for no less than 2 minutes [Note 1]. Centrifuge at 3500 rpm for 10 minutes [Note 2]. Carefully aspirate and discard the upper blue liquid as well as protein clots [Note 3]. Pipette 1 mL of the lower extract for color development [Note 4] [Note 5] [Note 6]. The detailed operating procedures are critical, please read the notes carefully.				
Lower extract (mL)	1.0	1.0	1.0	1.0
Chromogenic Reagent (mL)	0.2	0.2	0.2	0.2

Mix well and let stand for 5 minutes. Set zero with chromogenic at 550 nm using a 0.5 cm optical path cuvette [Note 7]. Perform parallel colorimetric determination on lipoprotein lipase tubes and hepatic lipase tubes, and measure the absorbance of each tube.

Notes: Detailed Operating Procedures

1. Perform thorough mixing and extraction for 2-5 minutes, timed with a stopwatch. Seal the test tube with a stopper or parafilm before extraction. During the extraction process, observe whether the liquid inside the tube is fully mixed evenly from top to bottom. While mixing, check if the color of the mixed solution is uniform throughout. If the color is darker in the upper layer and lighter in the lower layer, lift and lower the test tube repeatedly to assist mixing until the color of the mixed solution is consistent from top to bottom.

   After mixing for 2 minutes, observe whether the mixed solution undergoes stratification. If it separates into layers immediately with a transparent tube bottom, continue mixing until no immediate stratification occurs or the tube bottom presents a milky white state (except for the blank tube and standard tube).

2. A glass pipette must be used when aspirating the chloroform extract.
3. After extraction, centrifuge at 3500 rpm for 10 minutes. If the lower liquid is semi-solidified with a thick solidified layer or the volume of the lower liquid is less than 1 mL, gently stir the lower liquid with a small glass rod or epidural anesthesia needle core, then centrifuge again until clear layer separation is achieved.
4. Aspirate and discard the upper liquid and the solidified layer with a pipette.
5. Take a new syringe and epidural anesthesia needle core and sleeve (do not reuse those mentioned in Note 2 and Note 3). Before sampling each test tube, rinse the apparatus 2-3 times with absolute ethanol, then rinse another 2–3 times with chloroform extract. Insert the epidural anesthesia needle core into the needle sleeve, carefully insert it into the lower extract, pull out the needle core, attach the syringe, and aspirate 1.3-1.5 mL of the lower extract into another test tube.

   (This operation prevents the upper liquid and solidified layer from contaminating the lower extract; otherwise, the assay results will be affected.) If mist-like turbidity appears in the extract accidentally, place it in a 37°C water bath for 1-2 minutes, and the turbidity will

disappear.

6. Accurately pipette 1 mL of the lower extract from the above test tube into a new test tube, then add chromogenic reagent for color development.
7. Rinse the cuvette thoroughly with absolute ethanol first, then fill the cuvette with Reagent 6 for zero adjustment.
8. Only glass test tubes are allowed during the operation; plastic test tubes are not permitted as a substitute.

The above eight points are critical to the success of the experiment.

Calculation

1. Serum (Plasma)

Definition: One enzyme activity unit is defined as the production of 1 micromole (μmol) of free fatty acid (FFA) per milliliter per hour in the reaction system.

$$\text{LPL Activity (U/mL)} = \frac{A_{\text{LPL Assay}} - A_{\text{Blank}}}{A_{\text{Standard}} - A_{\text{Blank}}} \times C_{\text{Standard}} \times N \times \frac{60}{T} \div 1000$$

$$\text{HL Activity (U/mL)} = \frac{A_{\text{HL Assay}} - A_{\text{Blank}}}{A_{\text{Standard}} - A_{\text{Blank}}} \times C_{\text{Standard}} \times N \times \frac{60}{T} \div 1000$$

$$\text{Total Lipase Activity} = \text{LPL Activity} + \text{HL Activity}$$

2. Tissue

Definition: One enzyme activity unit is defined as the production of 1 micromole (μmol) of free fatty acid (FFA) per milligram of tissue protein per hour in the reaction system.

$$\text{LPL Activity (U/mg)} = \frac{A_{\text{LPL Assay}} - A_{\text{Blank}}}{A_{\text{Standard}} - A_{\text{Blank}}} \times C_{\text{Standard}} \times N \times \frac{60}{T} \div Cpr$$

$$\text{HL Activity (U/mg)} = \frac{A_{\text{HL Assay}} - A_{\text{Blank}}}{A_{\text{Standard}} - A_{\text{Blank}}} \times C_{\text{Standard}} \times N \times \frac{60}{T} \div Cpr$$

Total Lipase Activity = LPL Activity + HL Activity

Annotation:

$C_{Standard}$: Standard concentration, 500 μmol/L

N: Dilution multiple of the sample before testing

T: Reaction time, 20 min

Cpr: Protein concentration of homogenate (supernatant), mg/L

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.