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

Total lipase [Lipoprotein lipase (LPL)/Hepatic lipase (HL)] Activity Assay Kit

Catalog No.: BC00149

Size: 100T

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 [Lipoprotein lipase (LPL)/Hepatic lipase (HL)] Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Tissues, serum, plasma
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (optimal detection wavelength 550 nm)

Product Introduction

Lipoprotein lipase (LPL) and hepatic lipase (HL) break down triglycerides (TG) and hydrolyze them into glycerol and free fatty acids (FFA). The activities of LPL and HL can be calculated by measuring the amount of free fatty acids (FFA) produced using copper reagent. Hepatic lipase is a glycoprotein; its activity does not require activation by apolipoprotinin CII or certain ions, and it is not inhibited by high concentrations of salt or protamine. This characteristic allows for the separate calculation of the activities of LPL and HL.

Principle

Lipoprotein lipase (LPL) is present on the surface of capillary endothelial cells in extrahepatic tissues. It primarily catalyzes the hydrolysis of triglycerides (TG) in chylomicrons (CM) and very low-density lipoprotein (VLDL) in plasma, playing a crucial role in the degradation of CM and VLDL. Hepatic lipase (HL), on the other hand, is only present on the surface of liver endothelial cells and plays a vital role in the metabolism of intermediate-density lipoprotein (LDL) and high-density lipoprotein (HDL).

Components

Components	Size	Storage
Reagent 1	4 tubes of powder	2~8℃
	10 mL × 1	2~8℃
Preparation of Reagent 1: Dissolve each vial of powder in 1.5 mL of solvent and store at 4°C.		
Reagent 2	Accelerator, 6 mL × 2	2~8℃
	Inhibitor, 6 mL × 2	2~8℃
Reagent 3	6 mL × 2	2~8℃
Reagent 4	Solution A, 0.5 mL × 1	2~8℃
	60 mL × 1	2~8℃
Preparation of the substrate application solution for reagent four: Prepare the solution according to the ratio of solution A to solution B = 1:200, preparing only the required amount. After preparation, preheat the solution in a 37°C water bath for at least 10 minutes.		
Reagent 5	Solution A, 4 mL × 2	2~8℃
	Solution B, 4 mL × 1	2~8℃
	Solution C, 30 mL × 2	2~8℃
Preparation of copper reagent: Prepare the solution according to the ratio of solution A: solution B: solution C = 2:1:17. The prepared copper reagent should be a clear liquid. Prepare only the amount needed (do not mix unused reagent with newly prepared copper reagent, otherwise it will affect the results). Preheat the solution to 37°C for at least 10 minutes before use. Please strictly follow the order of adding solution A, B, and C, and mix thoroughly after each addition.		
Reagent 6	400 mL chloroform (to be prepared by yourself)	
Reagent 7	Nail powder, 4 mL × 3 tubes	2~8℃
	Solution B, 10 mL × 3 bottles	2~8℃
	Solution C, 0.5 mL × 2 tubes	2~8℃
Preparation of the color developer: Dissolve one ampoule of powder A in 10 mL of solution B thoroughly. Add solution C at a ratio of 100:1 1-2 minutes before use, mix well, and use immediately. Prepare only the amount needed. Do not reuse any unused color developer. The mixture of A and B can be stored at -20°C.		

Reagent 8	Standard, powder × 3	2~8℃
	Solvent, 60 mL × 1	2~8℃
Preparation of 2 mmol/L palmitic acid standard stock solution: Dissolve one vial of standard powder in solvent and bring the volume to 10 mL. Make sure to mix thoroughly.		
Preparation of 500 μmol/L palmitic acid standard: Take 2 mmol/L palmitic acid standard stock solution and dilute it 4 times with solvent, that is, 2 mmol/L palmitic acid standard: solvent = 1: 3 dilution.		

Storage

The kit has a shelf life of 3 months

Preparation before the experiment

Sample processing

1. Organization: Accurately weigh the sample and add 9 times the volume of physiological saline according to the ratio of weight (g): volume (mL) = 1:9. Mechanically homogenize under ice-water bath conditions to prepare a 10% homogenate. Centrifuge at 2500 rpm for 10 minutes and collect the supernatant for testing.
2. Serum (plasma): measured directly.

Operation process

Liquid Sample Handling Sheet:

Reagents(mL)	Blank tube	Standard tube	Lipoprotein lipase	Liver lipase
Distilled water	0.3	0.25	0.1	--
500 μmol/L standard solution	--	0.05	--	--
Sample	--	--	0.05	0.05
Reagent 1	--	--	0.05	0.05

Shake the test tube rack to mix.				
Reagent 2 Accelerator	--	--	--	0.1
Reagent 2 Inhibitor	--	--	0.1	--
	--	--	--	0.1
Shake the test tube rack to mix.				
Reagent 4 Substrate Application Solution (Preheat to 37℃)	0.5	0.5	0.5	0.5
Vortex mix thoroughly, then bathe in a 37 °C water bath for 20 minutes.				
Reagent 5 Copper Reagent (Preheat to 37℃)	0.5	0.5	0.5	0.5
Vortex mix for 10 seconds, then bathe in a 37 °C water bath for 10 minutes.				
Reagent: hexachloroform (Preheat to 37℃)	3.0	3.0	3.0	3.0
Incubate at 37°C for 10 minutes, then vortex for at least 2 minutes to fully mix and extract [Note 1]. Centrifuge at 500 rpm for 10 minutes [Note 2], carefully aspirate the upper blue liquid and protein clots [Note 3], and extract 1 mL of the lower layer for color development [Note 4] [Note 5] [Note 6]. Please read the notes carefully.				
Lower layer extract	1.0	1.0	1.0	1.0
Reagent 7, colorimetric reagent	0.2	0.2	0.2	0.2
Mix well, let stand for 5 minutes, and measure the absorbance of each tube at 550 nm with a 0.5 cm optical path. Zero the reagent with reagent 6 [Note 7]. Compare the lipoprotein lipase and liver lipase tubes in parallel and measure the absorbance of each tube.				

Note: Detailed operating steps

1. Mix thoroughly and extract for 2-5 minutes (timed with a stopwatch). Before extraction, seal the test tube with a stopper or film. During extraction, observe whether the liquid in the test tube is mixed evenly. While mixing, observe whether the color of the mixture is uniform. If the color is darker at the top and lighter at the bottom, lift the tube and then lower it to mix again until the color of the mixture is uniform. After mixing for 2 minutes, observe whether the mixture separates into layers. If it separates immediately and the

bottom of the tube is transparent, continue mixing until it does not separate immediately or the bottom of the tube is milky white (except for blank and standard tubes).

2. When drawing up the extract of reagent 6, a glass pipette must be used.
3. After extraction, centrifuge at 3500 rpm for 10 minutes. If the lower layer is found to be semi-solid, the solidified layer is thick, or the lower layer is less than 1 mL, gently stir the lower layer with a small glass rod or epidural needle core and centrifuge again until the layers are clearly separated.
4. Use a pipette to remove the upper liquid and solidified layer and discard them.
5. Take a separate syringe and epidural anesthesia needle core and sheath (do not use the syringes and needle core sheaths from Notes 2 and 3). Before drawing from each test tube, rinse it 2-3 times with anhydrous ethanol, then rinse it 2-3 times with reagent six extraction solution. Next, insert the epidural anesthesia needle core into the sheath and carefully insert it into the lower layer of the extract. Remove the core, connect the syringe, and draw 1.3-1.5 mL of the lower layer extract into another test tube. (This prevents the upper layer and solidified layer from mixing with the lower layer, which would affect the results.) If the extract is found to be cloudy, place it in a 37°C water bath for 1-2 minutes; the cloudiness should disappear.
6. Then, accurately pipette 1 mL of the lower layer extract from the above test tube and add it to another test tube, then add a colorimetric reagent to develop the color.
7. First, clean the cuvette with anhydrous ethanol, then pour reagent 6 into the cuvette and zero it.
8. Only glass tubes can be used in the experiment; plastic test tubes cannot be substituted. The above eight points are crucial to the success of the experiment.

Result calculation

1、Serum (plasma) calculation:

Unit definition: One micromole (μmol) of free fatty acid (FFA) produced per milliliter per hour in the reaction system is defined as one enzyme activity unit (FFA μmol/mL serum (plasma)•hour).

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

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

2、Tissue sample viability calculation:

Unit definition: One micromole (μmol) of free fatty acid (FFA) produced per milligram of tissue protein per hour in the reaction system is defined as one enzyme activity unit (FFA μmol/mgprot•hour).

$$\text{LPL activity（U/mL）} = \frac{A_{LPL} - A_{blank}}{A_{Standard} - A_{blank}} \times C_{Standard} \times N \times \frac{60}{T} \div Cpr$$

$$\text{HL activity（U/mL）} = \frac{A_{HL} - A_{blank}}{A_{Standard} - A_{blank}} \times C_{Standard} \times N \times \frac{60}{T} \div Cpr$$

Annotation

Total lipase activity = lipoprotein lipase activity + liver lipase activity;

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

N: Dilution factor of the sample before testing;

T: Reaction time, 20 min;

Cpr : Protein concentration in tissue homogenate , mgprot/L (prot refers to protein).

Precautions

This product is for research purposes only and must not be used for clinical diagnosis. Do not take it.