Lipoproteinlipase (LPL) Activity Assay Kit

Note: The reagents have been changed, so please be aware of and follow this instruction strictly.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC2445

Size: 100T/48S

Components:

Reagent I: 80 mL×1. Storage at 2-8℃.

Reagent II: Powder×1. Storage at 2-8℃, before use, add 1 mL acetone to fully dissolve it. The unused reagent can be stored at 2-8℃ for 4 weeks (One powder can be used for 100T after dissolution. In order to prolong the use time, this product is given one more powder).

Reagent III: 20 mL×1. Storage at 2-8℃.

Standard: 1 mL×1, 5 μmol/mL p-nitrophenol standard solution, stored at 2-8℃.

Product Description:

Lipoproteinlipase (LPL) is a rate-lowering enzyme for triglyceride degradation that catalyzes the hydrolysis of plasma triglycerides to fatty acids and monoglycerides, leading to the release of fatty acids from muscle and adipose tissues, and plays an important role in lipid metabolism and transport.

Lipoprotein esterase hydrolyzes 4-nitrophenyl palmitate to produce 4-nitrophenol, which has a characteristic absorption peak at 400 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate Reader, Low Temperature Centrifuge, Water-Bath, Micro Glass Cuvette/96 Well Flat-Bottom Plate, Transferpettor, Mortar/Homogenizer/Cell Ultrasonic Crusher, Ice, Acetone, Distilled Water.

Operation procedure:

I. Sample Preparation

1. Bacteria/cultured cells:

First collect bacteria or cells into the centrifuge tube and discard the supernatant after centrifugation. According to the number of bacteria or cells (10^4): the volume of Reagent I (mL) is 500-1000:1 (it is recommended to add 1 mL of Reagent I to 5 million bacteria/cells), ultrasound breaks bacteria/cells (ice bath, power 200W, ultrasound 3s, interval 10s, repeat 30 times). Centrifuge at 10000 ×g for 10 minutes at 4℃, take the supernatant and put it on ice for testing.

2. Tissue:

According to the mass of tissue (g): the volume of Reagent I (mL) of 1:5~10 (it is recommended to weigh about 0.1 g of tissue and add 1 mL of Reagent I), carry out ice bath homogenization. Centrifuge at 10000 ×g for 10 minutes at 4℃, take the supernatant and place it on ice for testing.

3. Serum sample:

Direct detection.

II. Detection

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 400 nm, set zero with distilled water.

2. Standard solution: Before use, take 30μL of 5μmol/mL standard solution and add 450μL of reagent I. Mix well and formulate into 0.3125μmol/mL standard solution for use. (In the experiment, 30μL is needed for each tube, so a large volume is prepared to minimize the experimental error.).

3. Operation table: carry out the following operations in 1.5 mL EP tupe:

Reagent Name (μL)	Contrast tube (C)	Test tube (T)	Standard tube (S)	Blank tube (B)
Sample	30	30	-	-
Standard solution	-	-	30	-
Distilled water	-	-	-	30
Reagent I	120	108	120	120
Reagent II	-	12	-	-
Mix well, water bath at 45℃ for 10 minutes.			-	-
Reagent III	150	150	150	150
After fully mixing and placing for 2 minutes, centrifugate at 8000 ×g of the contrast tube and the test tube at room temperature for 10 minutes. Take 200 μL of the supernatant of the contrast tube and the test tube, the standard tube and the blank tube to micro glass cuvette/96 well flat-bottom plate, measure the light absorption value at 400 nm, record as A_C, A_T, A_S, A_B, $\Delta A=A_T-A_C$, $\Delta A_S=A_S-A_B$.(The standard curve and blank tube only need to be measured 1-2 times.)				

III. LPL activity calculations

1. Serum

Unit definition: One unit of enzyme activity is defined as the amount of enzymes hydrolysis the generation of 1 nmol of 4-nitrophenol in the reaction system per minute at 45℃ and pH 7.5 every milliliter serum.

LPL (U/mL) $=\Delta A\div(\Delta A_S\div C_S)\div T\times1000 = 31.25\times\Delta A\div\Delta A_S$.

2. Tissues, bacteria or cells

(1) calculation by weight of sample

Unit definition: An enzyme activity unit is defined as 1nmol of 4-nitrophenol produced by hydrolysis per gram of tissues per minute at 45℃ and pH 7.5.

LPL (U/g weight) $=\Delta A\div(\Delta A_S\div C_S)\times V_E\div W\div T\times1000=31.25\times\Delta A\div\Delta A_S\div W$.

(2) Sample protein concentration

Unit definition: An enzyme activity unit is defined as 1nmol of 4-nitrophenol produced by hydrolysis per milligram of protein per minute at 45℃ and pH 7.5.

LPL (U/mg prot) $=\Delta A\div(\Delta A_S\div C_S)\times V_E\div(V_E\times Cpr)\div T\times1000=31.25\times\Delta A\div\Delta A_S\div Cpr$.

(3) Density of bacteria or cells:

Unit definition: An enzyme activity unit is defined as 1nmol of 4-nitrophenol produced by hydrolysis
per 10^4 cell per minute at 45°C and pH 7.5.

LPL ($U/10^4$ cell) =$\Delta A \div (\Delta A_S \div C_S) \times V_E \div N \div T \times 1000 = 31.25 \times \Delta A \div \Delta A_S \div N$.

C_S: Concentration of standard solution, 0.3125 μmol/mL;
V_E: Add the volume of Reagent I, 1 mL;
T: Reaction time, 10 minutes;
Cpr: Concentration of sample protein, mg/mL;
W: Sample mass, g;
N: Total number of bacteria/cells, 5 million;
1000: Unit conversion coefficient, 1 μmol = 1000 nmol.

Note:

1. After Reagent II is added to the test tube, it becomes turbid that is normal normal.
2. If A is greater than 2, dilute the crude enzyme solution with Reagent I and then determine.

Experimental example:

1. Take 0.1g rat muscle and add 1 mL of Reagent I, take it up and operate according to the measurement procedure. The calculation of ΔA= a tube a is calculated by 96 well plate. The results show that $\Delta A = A_T - A_C = 0.367 - 0.128 = 0.239$，$\Delta A_S = A_S - A_B = 0.378 - 0.046 = 0.332$，The enzyme activity is calculated according to the sample quality

LPL (U/g mass) = $31.25 \times \Delta A \div \Delta A_S \div W = 31.25 \times 0.239 \div 0.332 \div 0.1 = 224.96$ U/g mass.

2. The rabbit serum is directly operated according to the measurement procedure. The enzyme activity is calculated by 96 well plate, $\Delta A = A_T - A_C = 0.750 - 0.152 = 0.598$, $\Delta A_S = A_S - A_B = 0.378 - 0.046 = 0.332$, and the enzyme activity was calculated according to the volume of serum (plasma):

LPL (U/mL) =$31.25 \times \Delta A \div \Delta A_S \div W = 31.25 \times 0.598 \div 0.332 = 56.29$ U/mL.

Related products：

BC0590/BC0595	Free fatty Acids(FFA) Content Assay Kit
BC1080/BC1085	Alcohol Dehydrogenase(ADH) Activity Assay Kit
BC0320/BC0325	Plant Lipoxygenase(LOX) Activity Assay Kit