# Lipoproteinlipase (LPL) Activity Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer

**Catalog Number:** NA0696

**Size:**50T/24S

**Components:**

Reagent I: 60 mL×1. Storage at 4℃.

Reagent II: Powder×1. Storage at 4℃, protect from light. Dissolve it with 1.5 mL of acetone when the solution will be used.

Reagent III: 30 mL×1. Storage at 4℃.

Standard: 1 mL×1, 5 μmol/mL p-nitrophenol standard solution, stored at 4℃.

**Product Description:**

Lipoproteinlipase (LPL) is a speed reducing enzyme for the degradation of triglycerides. It can catalyzes the hydrolysis of triglycerides to fatty acids and monoglycerides. It is mainly synthesized in liver parenchymal cells and plays an important role in lipid metabolism and transport.

Lipoproteinlipase catalyzes the hydrolysis of 4-nitrophenylpalmitate to produce 4-nitrophenol with a characteristic absorption peak at 400 nm.

**Reagents and Equipment Required but Not Provided:**

Spectrophotometer, low temperature centrifuge, water-bath, 1 mL glass cuvette, transferpettor, mortar/homogenizer, EP tube, ice, distilled water.

**Operation procedure:**

**I. Sample Preparation**

1. Bacteria/cultured cells:

First collect bacteria/cells into the centrifuge tube and discard the supernatant after centrifugation. According to the number of bacteria/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 20%/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 for 30 minutes, adjust wavelength to 400 nm, set zero with distilled water.
2. Dilute the standard solution of 5 μmol/mL with Reagent I for 16 times to 0.3125 μmol/mL for standby.
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 | 100 | 100 | - | - |
| Standard solution | - | - | 100 | - |
| Distilled water | - | - | - | 100 |
| Reagent I | 400 | 360 | 400 | 400 |
| Reagent II | - | 40 | - | - |
| Mix well, water bath at 45℃ for 10 minutes. | | | - | - |
| Reagent III | 500 | 500 | 500 | 500 |
| After fully mixing and placing for 2 minutes, centrifuge at 8000 ×g of the contrast tube and the test tube at room temperature for 10 minutes. Take the supernatant of the contrast tube and the test tube, the standard tube and the blank tube to 1 mL glass cuvette, measure the light absorption value at 400 nm, record as $A_C$, $A_T$, $A_S$, $A_B$, $\Delta A=A_T-A_C$, $\Delta A_S=A_S-A_B$. | | | | |

**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 mL 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 fresh weight of sample

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 g sample.

LPL (U/g fresh 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: 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 mg protein.

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: 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 $10^4$ bacteria or cells.

LPL (U/$10^4$ cell) $=\Delta A\div(\Delta A_S\div C_S)\times V_E\div500\div T\times1000=0.0625\times\Delta A\div\Delta A_S$.

$C_S$: Concentration of standard solution, 0.3125 μmol/mL;

$V_E$: Add the volume of Reagent, 1 mL;

T: Reaction time, 10 minutes;

Cpr: Concentration of sample protein, mg/mL;
W: Sample mass, g;
500: 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.
2. If A is greater than 1, 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. After taking the supernatant, operate according to the determination steps. Calculate $\Delta A = A_T - A_C = 0.697-0.160 = 0.537$, $\Delta A_S = A_S - A_B = 0.545-0.001 = 0.544$
LPL(U/g mass) = $31.25 \times \Delta A \div \Delta A_S \div W = 31.25 \times 0.537 \div 0.544 \div 0.1 = 308.48$ U/g mass.
2. After the rabbit serum was diluted twice, the operation was carried out according to the determination steps. The results showed that Δ a = a test tube-a control tube = 0.639-0.096 = 0.543, Δ a standard = a standard tube-a blank tube = 0.545-0.001 = 0.544
LPL (U/mL) = $31.25 \times \Delta A \div \Delta A_S \times 2$ (dilution ratio) = $31.25 \times 0.543 \div 0.544 \times 2$ (dilution ratio) = 62.39 U/mL.

**Related Products：**

NA0822/NA0580 Free fatty Acids(FFA) Content Assay Kit
NA0790/NA0549 Alcohol Dehydrogenase(ADH) Activity Assay Kit
NA0842/NA0600 Plant Lipoxygenase(LOX) Activity Assay Kit