CheKine™ Micro Lipoprotein Lipase (LPL) Activity Assay Kit

Cat #: KTB2251 Size: 48 T/24 S 96 T/48 S

	Micro Lipoprotein Lipase (LPL) Activity Assay Kit		
REF	**Cat #:** KTB2251	LOT	**Lot #:** Refer to product label
	Detection range: 0.039-2.5 μmol/mL		**Sensitivity:** 0.039 μmol/mL
	Applicable samples: Animal Tissues, Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Lipoprotein lipase (LPL) is an enzyme synthesized by adipocytes, cardiomyocytes, skeletal muscle cells, mammary cells, macrophages and other parenchymal cells. LPL catalyzes the hydrolysis of triglyceride to fatty acids and monophosphate for tissue oxidation for energy supply and storage. Hydrolysis of 4-nitrobenzene palmitate by LPL yields 4-nitrophenol with a characteristic absorption peak at 400 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	71 mL	71×2 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅲ	14 mL	28 mL	4℃
Standard	1 mL	1 mL	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

·Microplate reader or visible spectrophotometer capable of measuring absorbance at 400 nm
·96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
·Water bath, Thermostat, ice machine, centrifuge, ultrasonic crusher
·Acetone
·Homogenizer or mortar (for tissue samples)

Reagent Preparation

Reagent I： Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent II： Prepared before use. 48 T add 1.25 mL deionized water, 96 T add 2.5 mL deionized water, fully dissolve. Inexhaustible reagents stored at -20℃ for 6 months, protected from light.

Reagent III： Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Standard： 5 μmol/mL p-nitrophenol solution,Store at 4℃, protected from light.

Note: Standard is toxic, so it is recommended to experiment in a fume hood.

Standard preparation： A standard solution of 5 μmol/mL p-nitrophenol was used and further diluted into standards as indicated in the table below.

序号	Standard volume	Reagent I volume（μL）	Concentration（μmol/mL）
Std.1	500 μL 5 μmol/mL Standard	500	2.5
Std.2	500 μL of Std.1 (2.5 μmol/mL)	500	1.25
Std.3	500 μL of Std.2 (1.25 μmol/mL)	500	0.625
Std.4	500 μL of Std.3 (0.625 μmol/mL)	500	0.313
Std.5	500 μL of Std.4 (0.313 μmol/mL)	500	0.156
Std.6	500 μL of Std.5 (0.156 μmol/mL)	500	0.078
Std.7	500 μL of Std.6 (0.078 μmol/mL)	500	0.039
Blank	0	500	0

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1.Animal Tissues: Weigh 0.1 g tissue, add 1 mL Reagent I and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

2.Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Reagent I to ultrasonically disrupt the cells 3 min (power 300 W, ultrasonic 3 s, interval 7 s). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

3.Plasma, Serum or other Liquid samples: Direct detection.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 400 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Control Well (μL)	Test Well (μL)	Standard Well (μL)	Blank Well (μL)
Sample supernatant	20	20	0	0

Standard	0	0	20	0
Reagent I	8	0	8	20
Reagent II	0	8	0	8
Mix well and Incubation with 10 min at 45℃			The following reagents were continued without incubation	
Reagent III	172	172	172	172
Mix thoroughly and stand for at 25℃ for 2 min, detect the absorbance at 400 nm was measured and recorded as $A_{Control}$, A_{Test}, $A_{Standard}$ and A_{Blank}, respectively. $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.				

Note: One Control Well was required for each Test Well, the Blank Well and the Standard curve only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.05, increase the sample quantity appropriately. If ΔA_{Test} is greater than 2, the sample can be appropriately diluted with Reagent I, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.Finally, the formula was modified when calculating the enzyme activity.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1.Drawing of the standard curve

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve, get the standard equation, and bring the ΔA_{Test} into the equation to get the x value (μmol/mL).

2.Calculation of Lipoprotein lipase activity:

（1）Calculated by sample protein concentration:

Active unit definition : At 45°C and pH 7.5 , the amount of enzyme required to decompose per mg protein to produce 1 nmol of 4-nitrophenol per minute is one unit of enzyme activity.

LPL(U/mg prot)=$x\times V_{extrac}\div(V_{extrac}\times Cpr)\div T\times 1{,}000$**$=100\times x\div Cpr$**

（2）Calculated by fresh weight of samples:

Active unit definition : At 45°C and pH 7.5 , the amount of enzyme required to decompose per mg protein to produce 1 nmol of 4-nitrophenol per minute is one unit of enzyme activity.

LPL(U/g fresh weight)=$x\times V_{extrac}\div W\div T\times 1{,}000$**$=100\times x\div w$**

（3）Calculated by cells:

Active unit definition : At 45°C and pH 7.5 , the amount of enzyme required to decompose per mg protein to produce 1 nmol of 4-nitrophenol per minute is one unit of enzyme activity.

LPL(U/10^4 cell)=$x\times V_{extrac}\div 500\div T\times 1{,}000$**$=0.2\times x$**

（4）Calculated by volume of liquid samples:

Active unit definition : At 45°C and pH 7.5 , the amount of enzyme required to decompose per mg protein to produce 1 nmol of 4-nitrophenol per minute is one unit of enzyme activity.

LPL(U/mL)=$x\div T\times 1{,}000$**$=100\times x$**

V_{extrac}: Added Reagent I total volume, 1 mL; Cpr : Sample protein concentration, mg/mL ; T : Reaction time, 10 min ; 1000 : Unit conversion coefficient , 1 μmol=1,000 nmol ; W : Sample weight , g ; 500 : The total number of cells ,5×10^6.

Points to note

1.The turbidity of the Test Wells after adding reagent II was a normal phenomenon.

Typical Data

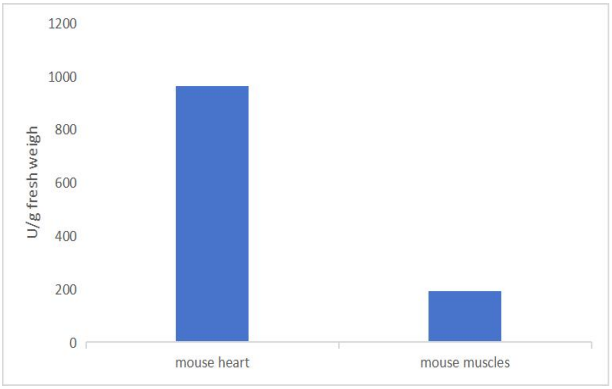

Figure 1.Determination of LPL activity in mouse heart and mouse muscle by this kit.

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-glucosidase(α-GC) Activity Assay Kit
KTB1121	CheKine™ Micro Pyruvate Acid (PA) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.