

LPL ELISA
Product No. 231049

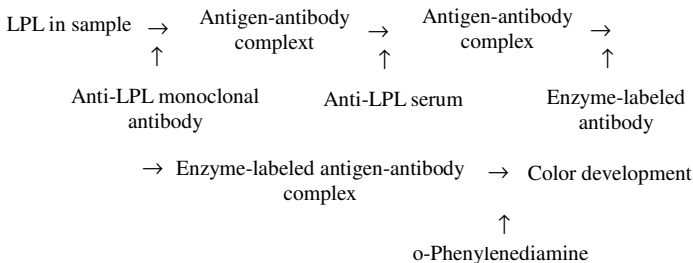
Storage: 2–10°C For Research Use Only!

INTENDED USE

Measurement of lipoprotein lipase in serum or plasma
Lipoprotein lipase (LPL) is an enzyme that hydrolyzes triglyceride (TG) of chylomicron (CM) synthesized in the small intestine by a metabolic pathway of food and very low density lipoprotein (VLDL) synthesized in the liver by an endogenous metabolic pathway.
Deficiency or dysfunction of LPL is observed in patients with hyperlipidemia of types I, IV and V, therefore, the LPL decrease is considered as one cause of hypertri- glyceridemia.

ASSAY PRINCIPLE

Lipoprotein lipase (LPL) in samples is trapped on the plate due to the antigen-antibody reaction with anti-bovine milk lipoprotein lipase mouse monoclonal antibody (anti-LPL monoclonal antibody) coated on the plate. Subsequently, anti-bovine milk lipoprotein lipase chicken serum (anti-LPL serum) is added, and then Horseradish peroxidase-labeled anti-chicken immunoglobulin G goat serum (enzyme-labeled antibody) is added to initiate antigen-antibody reactions in each serum. After that, the substrate, o-phenylenediamine, is added for color development. The absorbance of the developed color is measured to determine LPL levels.



Features:

- 1) Hepatic lipase effect is eliminated by using a highly specific monoclonal antibody.
- 2) High sensitivity is achieved.
- 3) Simple operations exhibit good reproducibility.
- 4) Assays are not affected by coexisting substances.

DESCRIPTION (KIT COMPONENTS)

Constituent Reagents	Ingredients and Quantity
MTP	LPL-mAb Coated Plate: Binding wells for anti-bovine milk lipoprotein lipase mouse monoclonal antibody
WASH	Wash Buffer Concentrate: Surfactant
DILB	Dilution Buffer: Phosphate buffer (pH 7.2) 25 mmol/L
PAP	Anti-LPL pAb (lyophilized): Anti-bovine milk lipoprotein lipase chicken serum
CON	Enzyme-labeled pAb Concentrate: Horseradish peroxidase-labeled anti-chicken immunoglobulin G goat serum
SUB	Substrate (lyophilized): o-Phenylenediamine dihydrochloride
SUBB	Substrate Buffer: Hydrogen peroxide
STOP	Stop Reagent: Sulfuric acid (7.7%)
STD	Standard (lyophilized): Human plasma

PRECAUTIONS FOR USE

1. Sample Properties and Sampling Methods

- i) Samples
Serum or heparin plasma can be used.
(EDTA plasma and citrate plasma cannot be used.)

When 30 or 50 IU/kg body weight of heparin (JP heparin sodium injection) is intravenously injected, the maximum blood LPL level is achieved 10-15 minutes after the injection.²⁾ The dose of heparin and the blood collection time should be identical for several assays.

ii) Storage of Samples

The isolated serum (plasma) should be tested on the same day. Otherwise, store it at -20°C or below. Note that the assay should be conducted after the sample reached room temperature (15-30°C).

2. Interfering Substances

Assay will not be affected by free bilirubin less than or equal to 20 mg/dL, conjugated bilirubin less than or equal to 20 mg/dL, hemoglobin less than or equal to 500 mg/dL and Intralipos less than or equal to 5%.

3. Others

Always use the Calibrator Lyophilized in this kit as the Reference Standard.

USE AND DOSAGE (ASSAY PROCEDURES)

1. Preparation of Reagents

[MTP] LPL-mAb Coated Plate:
Ready to use.
Unused wells are stable for 2 weeks after seal when air-tightly stored at 2-10°C.

[WASH] Wash Buffer Solution:
Add purified water to the Wash Buffer Concentrate to make a volume of 1000 mL. The Wash Buffer Solution is stable for 2 weeks at 2-10°C.

[PAP] Anti-LPL pAb Solution:
Reconstitute the anti-LPL pAb Lyophilized by adding 6 mL of the Dilution Buffer. The anti-LPL pAb Solution is stable for 2 weeks at 2-10°C.

[CON] Enzyme-labeled pAb Solution:
Dilute the Enzyme-labeled pAb Concentrate with 6 mL of the Dilution Buffer. The Enzyme-labeled pAb Solution is stable for 2 weeks at 2-10°C.

[SUB] Substrate Solution:
Just prior to use, reconstitute the Substrate Lyophilized in 6 mL of the Substrate Buffer. This solution is prepared when needed.

[STOP] Stop Reagent:
Ready to use.

[STD] Standard Solution:
Reconstitute the lyophilized Standard by adding 1.0 mL of the Dilution Buffer (DILB). Dilute the solution in accordance with the following example to prepare the diluted Standard Solution (STD).
The undiluted Standard Solution is stable for 1 week stored at 2-10°C.

[Example]

Conc (ng/mL)	25	12.5	6.25	3.125	1.562	0.781	0.390	0
STD (μL)	As required	→150	→150	→150	→150	→150	→150	→0
DILB (μL)	0	150	150	150	150	150	150	As required

2. Pretreatment of Samples

Dilute the isolated serum or plasma to 1:20. For example, add 400 μL of the Dilution Buffer to 20 μL of serum to make the concentration 1:20.

3. Assay Procedures

Assay Method

- 1) Dispense 50 μL of the sample solution or Standard Solution to each well on the LPL-mAb Coated Plate. Mix the solution with a plate mixer and then allow to stand for 1 hour at 15-25°C. Note that duplicate assays are required for the Standard Solution.
- 2) Remove the solution from the wells, and wash inside with 0.35 mL of the Wash Buffer Solution for three times using a plate washer or manually.
- 3) Remove the Wash Buffer Solution, and add 50 μL of the Anti-LPL pAb Solution and then allow to stand for 30 minutes at 15-25°C.
- 4) Remove the solution from the wells, and wash inside with 0.35 mL of the Wash Buffer Solution for three times as Step 2).
- 5) Remove the Wash Buffer Solution, and add 50 μL of the Enzyme-labeled pAb Solution and then allow to stand for 30 minutes at 15-25°C.
- 6) Remove the solution from the wells, and wash inside with 0.35 mL of the Wash Buffer Solution for three times as Step 2).
- 7) Remove the Wash Buffer Solution, and add 50 μL of the Substrate Solution and then allow to stand for exactly 15 minutes at 15-25°C.
- 8) Add 50 μL of the Stop Reagent to terminate the reaction.
- 9) Read absorbance of the reactant at wavelength of 492nm using a microplate reader against a reagent blank as the control.

Calculation Method

- 1) In a double logarithmic plot, use the X-axis for the concentration of lipoprotein lipase and the Y-axis for absorbance. Plot the parameters for the Standard Solution and fit the data points to a smooth curve. This curve is designated the calibration curve.
- 2) Read the concentration value corresponding to the measured absorbance of the sample on the calibration curve. The readout multiplied by the dilution factor provides the concentration of lipoprotein lipase.

Precautions for Use

- 1) Use the Dilution Solution rather than the sample solution as a reagent blank.
- 2) A calibration curve should be prepared before each assay based on duplicate assays.
- 3) When measuring multiple samples, pay attention to the duration of each step so that an identical reaction time is used for wells.
- 4) Remove the Wash Buffer Solution completely after the washing step.
- 5) Place the sample solution, the Standard and each reagent in the center of each well avoiding contact on the well wall.
- 6) If the concentration of a sample exceeds the range of assay, dilute the sample with the Dilution Solution, and repeat the assay.

ASSESSMENT OF ASSAY RESULTS

1. Reference Interval
Before heparin injection: 45-63 ng/mL⁶⁾
After heparin injection: 164-284 ng/mL^{3)*}
*Note that this range is for plasma lipoprotein lipase in healthy patients whose samples are collected 15 minutes after an intravenous injection of 30 units/kg body weight of heparin (JP heparin sodium injection).
2. There may be reactions with non-target substances or interfering reactions. If assay results seem to be unreliable, repeat the assay (if necessary after dilution), or try another analytical assay.

PERFORMANCES

1. Sensitivity
 - 1) Reagent blank: Absorbance ≤ 0.2
 - 2) Sensitivity: Absorbance 0.3-1.4 per 10 ng/mL of lipoprotein lipase
2. Accuracy: 85-115% of the expected assay level
3. Within-run Reproducibility
Coefficient of variation of $\leq 10\%$
(The test method used for 1.-3. is that of Sekisui Medical Co., Ltd.)
4. Assay Range
4-500 ng/mL
5. Correlation⁷⁾
 - 1) Serum
N=54, $r=0.992$, $y=0.97x-1.3$
Control method: Comparison with plasma collected with this kit at the same time as serum
 - 2) Plasma
N=50, $r=0.986$, $y=0.95x+5.65$
Control method: Approved in vitro diagnostics (Enzyme immunoassay)
6. Reference Standard
Purified lipoprotein lipase (reference standard of Sekisui Medical Co., Ltd.)

PRECAUTIONS FOR USE AND HANDLING

1. Precautions for Handling (Risk Prevention)
 - 1) Remember that the samples can cause HIV, HBV and HCV infections. To eliminate the risk of infection, use disposable gloves and avoid mouth pipetting during the test.
 - 2) The lyophilized Standard was prepared from plasma that had been confirmed to be negative for HBs antigen, HCV antibody and HIV antibody. However, handle it with due care using gloves in recognition of potential infection similar to that of the sample.
 - 3) Sodium azide has been added as an antiseptic agent to the lyophilized anti-LPL pAb and the lyophilized standard. Therefore, if the reagents come in contact with the eyes, mouth or skin, rinse immediately with plenty of water as first aid, and consult a doctor if necessary.

- 4) Proclin 300, which possesses skin-irritative potential, has been added as an antiseptic agent in the Wash Buffer Concentrate, Dilution Buffer and Enzyme-labeled pAb Concentrate. Therefore, in case of contact with the skin or clothes, rinse immediately with plenty of water, and consult a doctor if skin irritation develops.
- 5) Sulfuric acid has been added to the Stop Reagent. Therefore, prevent the reagent from coming in contact with the eyes or skin. In case of eye contact rinse immediately with running water as first aid, and consult a doctor. In case of contact with the skin or clothing, rinse immediately with water.

2. Precautions for Use

- 1) This product should be stored as directed, and avoided freezing. Freezing can cause deterioration of the reagents, which can produce inaccurate results. Therefore, avoid using reagents that have previously been frozen.
- 2) Do not use a reagent that is past its shelf life. If such reagents are used, the reliability of assay values cannot be guaranteed.
- 3) Do not replenish reagents during the test.
- 4) Avoid direct sunlight during the test.

3. Precautions for Disposal

- 1) Before disposal, the remaining samples and sample containers etc. must be soaked in sodium hypochlorite solution with a concentration of greater than 0.1% for more than an hour or autoclaved at 121°C for 20 min.
- 2) If a sample or a solution containing sample is spilled, wipe it with such agents as sodium hypochlorite solution with a concentration of greater than 0.1% to prevent infection.
- 3) Before disposal, the reagents and treated samples etc. should be segregated into different types of waste, separated from medical waste and industrial waste, according to the waste disposal regulations.
- 4) The disposal of the reagents should be in consideration of such regulations as the Water Pollution Control Law.
- 5) Sodium azide has been added as an antiseptic agent to the lyophilized anti-LPL pAb Lyophilized and the lyophilized standard. It can react with lead or copper pipes to produce highly explosive metal azide. Therefore, the reagent should only be flushed with large amounts of water during disposal.

4. Other Precautions

Do not use the containers for any other purposes.

STORAGE AND SHELF LIFE

1. Storage temperature: 2-10°C
2. Shelf life: 2 years from the date of manufacture
(The expiration date is printed on the outside of the package.)

PACKAGE UNIT

	Reagent	Package
MTP	LPL-mAb coated plate	1 plate
WASH	Wash buffer concentrate (10x)	100 mL
DILB	Dilution buffer	100 mL
PAB	anti-LPL pAb (lyophilized)	1 vial
CON	Enzyme-labeled pAb concentrate (7x)	1 mL (7x)
SUB	Substrate (lyophilized)	2 vials
SUBB	Substrate buffer	15 mL
STOP	Stop reagent	10 mL
STD	Standard (lyophilized)	1 vial

REFERENCES

- 1) Kouji Shirai: Modern Medical Laboratory 21 (3), 211 (1993)
- 2) Kobayashi J. et al.: Clin. Chim. Acta. 216, 113 (1993)
- 3) Watanabe H. et al.: Atherosclerosis 145 (1), 45 (1999)
- 4) Kouji Shirai: Domyakukouka 25 (8), 309 (1998)
- 5) Saito K. et al.: Ann. Clin. Biochem. 35, 733 (1998)
- 6) Shirai K. et al.: Lipoprotein Metabolism and Atherogenesis 51 (2001)
- 7) Sekisui Medical Co., Ltd., company internal data