Code No. 27184

Human Lipoprotein Lipase (LPL) Assay Kit - IBL

INTRODUCTION

Lipoprotein lipase (LPL) is an enzyme involved in the metabolism of triglyceride-rich lipoproteins including chylomicron (CM) and very low-density lipoprotein (VLDL). Secreted LPL mainly binds to heparan sulfate at the luminal surface of the capillary endothelium and hydrolyzes the triglyceride (TG) in triglyceride-rich lipoprotein into fatty acids and monoglycerides. Therefore, deficiency and reduction in activity of LPL are considered one of the important causes of hypertriglyceridemia (Ref. 1 - 3).
For determination of LPL concentration in human blood samples, post-heparin plasma has been used mainly and that is a burden to person being tested (Ref. 4 - 6).
This ELISA kit can also measure small amount of circulating LPL in pre-heparin blood samples.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of highly specific antibodies. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is proportional to the quantities of Human LPL.

MEASUREMENT RANGE

0.04 - 2.8 ng/mL

INTENDED USE

For research use only, not for use in diagnostic procedures.

This IBL's assay kit is capable for the quantitative determination human LPL in serum, EDTA-plasma and cell culture supernatant.

KIT COMPONENT

1	Precoated plate : Anti-Human LPL (57A5) Mouse IgG MoAb Affinity Purify	96Well x 1
2	Labeled antibody Conc. : (30X) HRP conjugated Anti- Human LPL (88B8) Mouse IgG MoAb Fab' Affinity Purify	0.4mL x 1
3	Standard : Recombinant human LPL	1.0mL x 1
4	EIA buffer	30mL x 1
5	Solution for Labeled antibody*	12mL x 1
6	Chromogen : TMB solution	15mL x 1
7	Stop solution*	12mL x 1
8	Wash buffer Conc.*	50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

- Plate reader (450nm)
- Micropipette and tip
- Graduated cylinder and beaker
- Deionized water
- Refrigerator (as 4°C)
- Graph paper (log/log)
- Paper towel
- Tube for dilution of Standard
- Incubator (37°C ± 1°C)
- Washing bottle for precoated plate
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"

2. Preparation

1) Preparation of wash buffer
"8, Wash buffer Conc." is a concentrated (40X) buffer. Adjust the temperature of "8, Washing buffer Conc." to room temperature and then, mix it gently and completely before use. Dilute 50 mL of "8, Wash buffer Conc." with 1,950 mL of deionized water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.

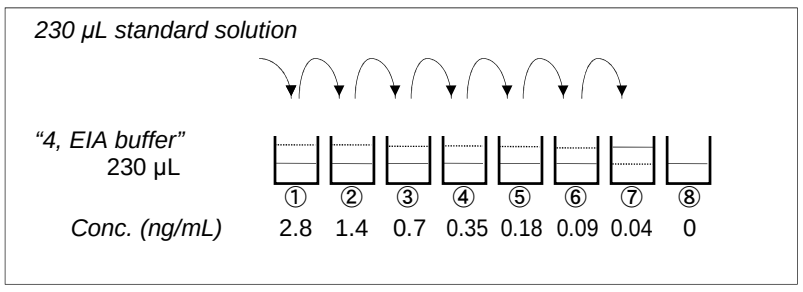
2) Preparation of Labeled antibody
"2, Labeled antibody Conc." is a concentrated (30X). Dilute "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.
Example)
In case you use one strip (8 well), the required quantity of Labeled antibody is 800 µL. (Dilute 30 µL of "2, Labeled antibody Conc." with 870 µL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 µL in each well.)
<u>This operation should be done just before applying labeled antibody.</u>
The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.

3) Dilution of Standard
"3, Standard" is 5.6 ng/mL standard solution of Human LPL.
Prepare 8 tubes for dilution and put 230 µL each of "4, EIA buffer" into the tube.
Specify the following concentration of each tube."

Tube-1	2.8 ng/mL
Tube-2	1.4 ng/mL
Tube-3	0.7 ng/mL
Tube-4	0.35 ng/mL
Tube-5	0.18 ng/mL
Tube-6	0.09 ng/mL
Tube-7	0.04 ng/mL
Tube-8	0 ng/mL (Test Sample Blank)

Put 230 µL of the standard solution into tube-1 and mix it gently. Then, put 230 µL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 2.8 ng/mL and 0.04 ng/mL. Tube-8 is the test sample blank as 0 ng/mL.
The remaining "3, Standard" must be stored at 4°C in firmly sealed vial until next using. Don't freeze it.

See following picture.



4) Dilution of test sample
Test samples have to be diluted with "4, EIA buffer" suitably.
If the concentration of Human LPL in samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.

Guide line of dilution for blood samples (serum and EDTA-plasma) of normal persons are as follows.
Pre-heparin: more than 100-fold
Post-heparin: more than 300-fold
When an abnormally low value is suspected, it is recommended to begin with doubling dilution.

3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Make sure of no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

Reagents	Test Sample	Standard	Test Sample Blank	Reagent Blank
	Test sample 100 µL	Diluted standard (Tube 1-7) 100 µL	EIA buffer (Tube-8) 100 µL	EIA buffer 100 µL
Incubation for 60 minutes at 37°C with plate lid				
4 times (wash buffer more than 350 µL) *				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 30 minutes at 4°C with plate lid				
5 times (wash buffer more than 350 µL) *				
Chromogen	100 µL	100 µL	100 µL	100 µL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 µL	100 µL	100 µL	100 µL
Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.				

1) Determine wells for reagent blank. Put 100 µL each of "4, EIA buffer" into the wells.
2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 µL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1-7) into the appropriate wells.
3) Incubate the precoated plate for 60 minutes at 37°C after covering it with plate lid.
4) Wash the plate with the prepared wash buffer and remove all liquid. *
5) Pipette 100 µL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
6) Incubate the precoated plate for 30 minutes at 4°C after covering it with plate lid.
7) Wash the plate with the prepared wash buffer and remove all liquid. *
8) Take the required quantity of "6, Chromogen" and put it into a disposable test tube. Then, pipette 100 µL from the test tube into every well. Please do not return the rest of used chromogen in the test tube into "6, Chromogen" bottle in order to avoid contamination.
9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The solution of Chromogen will turn blue.
10) Add 100 µL of "7, Stop solution" to all wells. Mix the solution by tapping the side of precoated plate. The solution will turn yellow by addition of "7, Stop solution".
11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the solution. Then, run the plate reader and conduct measurement at 450 nm against a reagent blank.
The measurement shall be done within 30 minutes after addition of "7, Stop solution".

SPECIAL ATTENTION

1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2) Test sampleshave to be diluted with "4, EIA buffer", suitably.
3) Duplicate measurement of test samples and standard is recommended.
4) Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
5) Use only wash buffer in this kit for washing the precoated plate.
Insufficient washing may lead to the failure in measurement.
6) Remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
7) "6, Chromogen" should be stored in the dark due to its sensitivity against light. Avoid contact of Chromogen with metals.

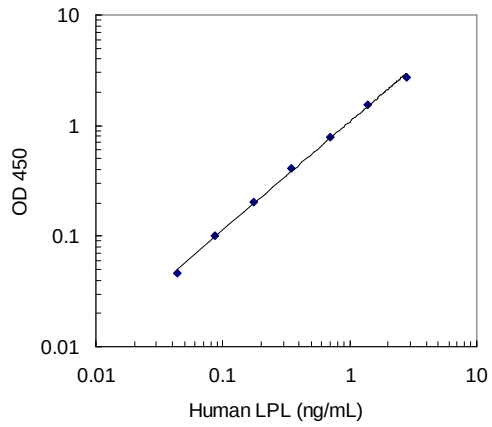
8) Measurement should be done within 30 minutes after addition of “7, Stop solution”.

CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve

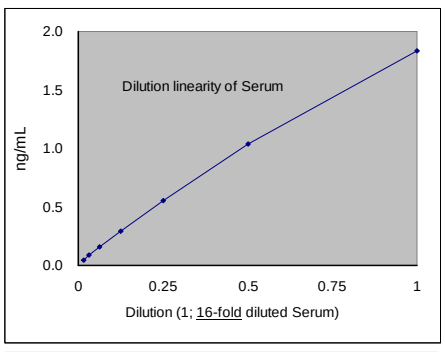
Conc. (ng/mL)	Absorbance (450nm)
2.8	2.723
1.4	1.559
0.7	0.798
0.35	0.412
0.18	0.210
0.09	0.104
0.04	0.051
0 (Test Sample Blank)	0.004

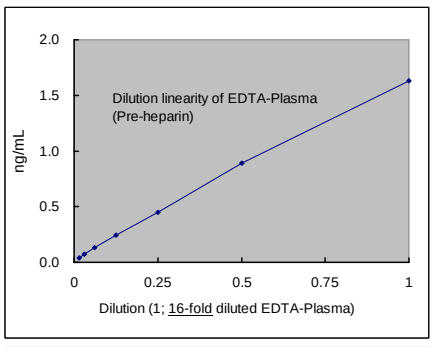


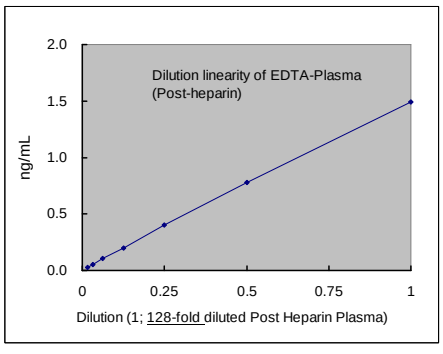
* The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

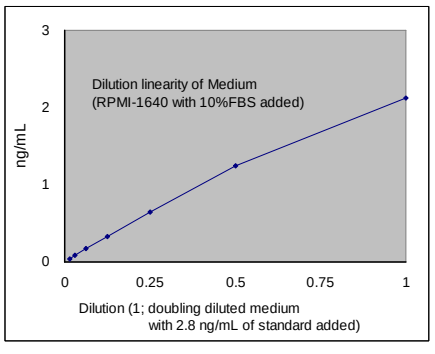
PERFORMANCE CHARACTERISTICS

1. Dilution linearity









2. Added Recovery Assay

Specimen	Additive Amount (ng/mL)	Theoretical Value (ng/mL)	Measured Value (ng/mL)	%
Human Serum (Pre-heparin) (x32)	0.35	2.612	2.432	93.1
	0.088	2.350	2.317	98.6
	0.011	2.273	2.149	94.5
Human Plasma (EDTA) (Pre-heparin) (x32)	0.35	2.373	2.240	94.4
	0.088	2.111	1.934	91.6
	0.011	2.034	2.001	98.4
Human Plasma (EDTA) (Post-heparin) (x256)	0.35	1.950	1.835	94.1
	0.088	1.687	1.607	95.3
	0.011	1.611	1.510	93.7
Medium with 10% FBS (x2)	2.8	2.800	2.093	74.7
	0.7	0.700	0.625	89.3
	0.088	0.088	0.080	90.9

3. Intra - Assay

Mean Value (ng/mL)	SD (ng/mL)	CV (%)	n
1.269	0.016	1.3	24
0.344	0.007	2.0	24
0.101	0.002	2.0	24

4. Inter - Assay

Mean Value (ng/mL)	SD (ng/mL)	CV (%)	n
1.309	0.019	1.5	9
0.354	0.004	1.1	9
0.106	0.001	0.9	9

5. Specificity

Substance	Cross-Reactivity
Human LPL	100 %
Human HTGL	< 0.1 %
Human EL	< 0.1 %

6. Sensitivity

0.009 ng/mL
The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.)

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. Do not freeze “3, Standard” solution.
3. “7, Stop solution” is a strong acid substance. Therefore, be careful not to have your skin and clothes contact “7, Stop solution” and pay attention to the disposal of “7, Stop solution”.
4. Dispose used materials after rinsing them with large quantity of water.
5. Precipitation may occur in “2, Labeled antibody Conc.”, “4, EIA buffer” or “8, Wash buffer Conc.”, however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from a different lot or kit.
8. Do not use expired reagents.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8°C
The expiry date is specified on outer box.

REFERENCE

1. Lipoprotein lipase: from gene to obesity. Wang H, Eckel RH. Am J Physiol Endocrinol Metab. 2009 Aug;297(2):E271-88.
2. A mutation in the promoter of the lipoprotein lipase (LPL) gene in a patient with familial combined hyperlipidemia and low LPL activity. Yang WS, Nevin DN, Peng R, Brunzell JD, Deeb SS. Proc Natl Acad Sci U S A. 1995 May 9;92(10):4462-6.
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4. A novel method for measuring human lipoprotein lipase and hepatic lipase activities in postheparin plasma. Imamura S, Kobayashi J, Nakajima K, Sakasegawa S, Nohara A, Noguchi T, Kawashiri MA, Inazu A, Deeb SS, Mabuchi H, Brunzell JD. J Lipid Res. 2008 Jul;49(7):1431-7.
5. Pre-heparin lipoprotein lipase mass. Kobayashi J. J Atheroscler Thromb. 2004;11(1):1-5.
6. Serum lipoprotein lipase mass: clinical significance of its measurement. Kobayashi J, Nohara A, Kawashiri MA, Inazu A, Koizumi J, Nakajima K, Mabuchi H. Clin Chim Acta. 2007 Mar;378(1-2):7-12.

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Made in Japan.