Product Manual

Lipoprotein Lipase (LPL) ELISA Kit

Catalog Number

STA- 611 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Triglycerides (TAG) are a type of lipid in the blood serving as an energy source and playing a key role in metabolism. Triglycerides are the digestive end product of breaking down dietary fats. Any extra carbohydrates and fats that are not immediately used are chemically converted into triglycerides. In the intestines, secreted enzyme lipases hydrolyse the triglyceride ester bond, yielding glycerol and free fatty acids in a process called lipolysis. Enterocytes then absorb and repackage the fragments with cholesterol to form chylomicrons, a major lipoprotein transport particle. In the liver, hepatic lipases also break down triglycerides to assemble another lipoprotein particle (VLDL) from triglycerides, cholesterol, and apolipoproteins. Plasma triglyceride levels are regulated by the assembly and degradation of VLDL and chylomicron particles. Lipoprotein lipase (LPL) is the key plasma lipase responsible for hydrolysis of the triglyceride core in these particles. A mutation in the gene coding for LPL can cause reduced LPL levels (LPL deficiency), wherein the body does not produce sufficient LPL necessary for fatty acid breakdown. LPL deficiency results in elevated levels of chylomicrons and is also known as chylomicronemia or Type Ia hyperlipoproteinemia.

Cell Biolabs' Lipoprotein Lipase (LPL) ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of lipoprotein lipase in plasma, serum or other biological fluid samples. The kit detects LPL from human, cow, rat, guinea pig, chicken, but not mouse samples, and has a detection sensitivity limit of 20 ng/mL LPL. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
2. STA-390: Total Cholesterol Assay Kit
3. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
4. STA-396: Serum Triglyceride Quantification Kit (Colorimetric)
5. STA-397: Serum Triglyceride Quantification Kit (Fluorometric)
6. STA-398: Free Glycerol Assay Kit (Colorimetric)
7. STA-399: Free Glycerol Assay Kit (Fluorometric)
8. STA-610: Lipoprotein Lipase (LPL) Activity Assay Kit (Fluorometric)

Kit Components

Box 1 (shipped at room temperature)

1. Anti-LPL Antibody Coated Plate (Part No. 261101): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-LPL Antibody (1000X) (Part No. 261102): One 15 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.

6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. LPL Standard (Part No. 261103): One 25 µL vial of 0.1 mg/mL Bovine LPL in 3.8 M Ammonium Sulfate, 20 mM Tris pH 7.5.

Materials Not Supplied

1. Plasma, Serum, or Other Biological Fluids
2. PBS containing 0.1% BSA
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the LPL Standard at -80ºC to avoid multiple freeze/thaw cycles. Store the remainder of the kit at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-LPL Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-LPL antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:4000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of LPL standards in the concentration range of 0 to 500 ng/mL in PBS containing 0.1% BSA (Table 1).

Standard Tubes	0.1 mg/mL LPL Standard (µL)	PBS containing 0.1% BSA (µL)	LPL (ng/mL)
1	5	995	500
2	500 of Tube #1	500	250
3	500 of Tube #2	500	125
4	500 of Tube #3	500	62.5
5	500 of Tube #4	500	31.25
6	500 of Tube #5	500	15
7	500 of Tube #6	500	7.5
8	0	500	0

Table 1. Preparation of LPL Standards.

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

- Plasma: Collect blood with heparin or EDTA and centrifuge for 10 minutes at 1000 g at 4ºC. Remove the plasma and assay immediately or store samples at -80ºC for up to three months. Normal plasma samples require about 2 to 10 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA.
- Serum: Harvest serum and centrifuge for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Normal serum samples require about 2 to 10 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA.
- Other Biological Fluids: Centrifuge samples for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months.

Assay Protocol

1. Prepare dilutions of plasma, serum, or other biological fluid samples in PBS containing 0.1% BSA as indicated in Preparation of Samples section above.
2. Add 100 µL of human LPL unknown sample or standard to the Anti-LPL Antibody Coated Plate. Each LPL unknown sample, standard and blank should be assayed in duplicate.
3. Incubate at 37ºC for at least 2 hours or 4ºC overnight.
4. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Biotinylated Anti-LPL antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
6. Wash the strip wells 3 times according to step 4 above.
7. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
8. Wash the strip wells 3 times according to step 4 above. Proceed immediately to the next step.
9. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

10. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
11. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Lipoprotein Lipase (LPL) ELISA Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

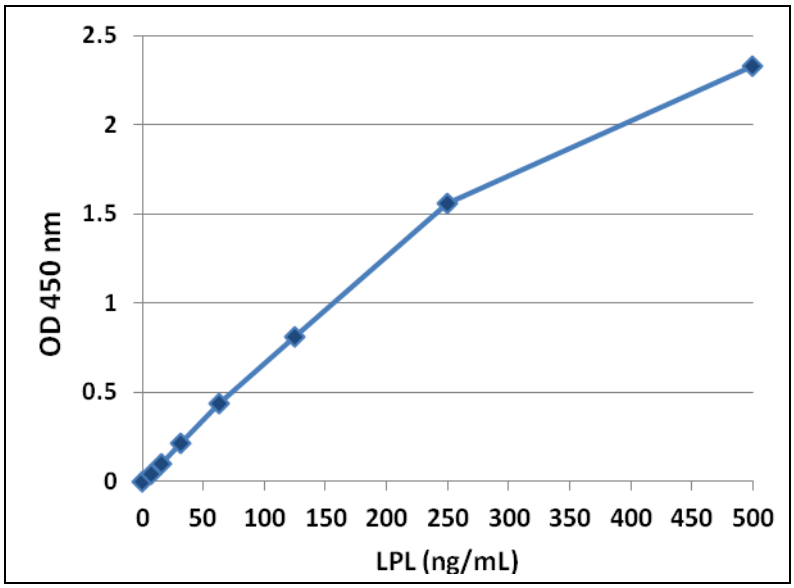

Figure 1: Lipoprotein Lipase (LPL) Standard Curve.

References

1. Goldberg, I. J., and M. Merkel (2001) *Front Biosci* **6**: D388-405.
2. Olivecrona, G., and T. Olivecrona (2010) *Curr Opin Lipidol* **21**: 409-415.
3. Wang, H., and R. H. Eckel (2009) *Am J Physiol Endocrinol Metab* **297**: E271-288.

Recent Product Citations

1. Garg, M. et al. (2017). Liraglutide acutely suppresses glucagon, lipolysis and ketogenesis in type 1 diabetes. *Diabetes Obes Metab*. doi: 10.1111/dom.12944.
2. Rhee, Y. H. & Ahn, J. C. (2016). Melatonin attenuated adipogenesis through reduction of the CCAAT/enhancer binding protein beta by regulating the glycogen synthase 3 beta in human mesenchymal stem cells. *J Physiol Biochem.* doi:10.1007/s13105-015-0463-3.

3. Chan, D. C. et al. (2014). Inter-relationships between proprotein convertase subtilisin/kexin type 9, apolipoprotein C-III and plasma apolipoprotein B-48 transport in obese subjects: a stable isotope study in the postprandial state. *Clin Sci (Lond)*. **128**:379-385.

Warranty

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