Product Manual

Lipoprotein Lipase (LPL) Activity Assay Kit (Fluorometric), Trial Size

Catalog Number

STA- 610- T **20 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.

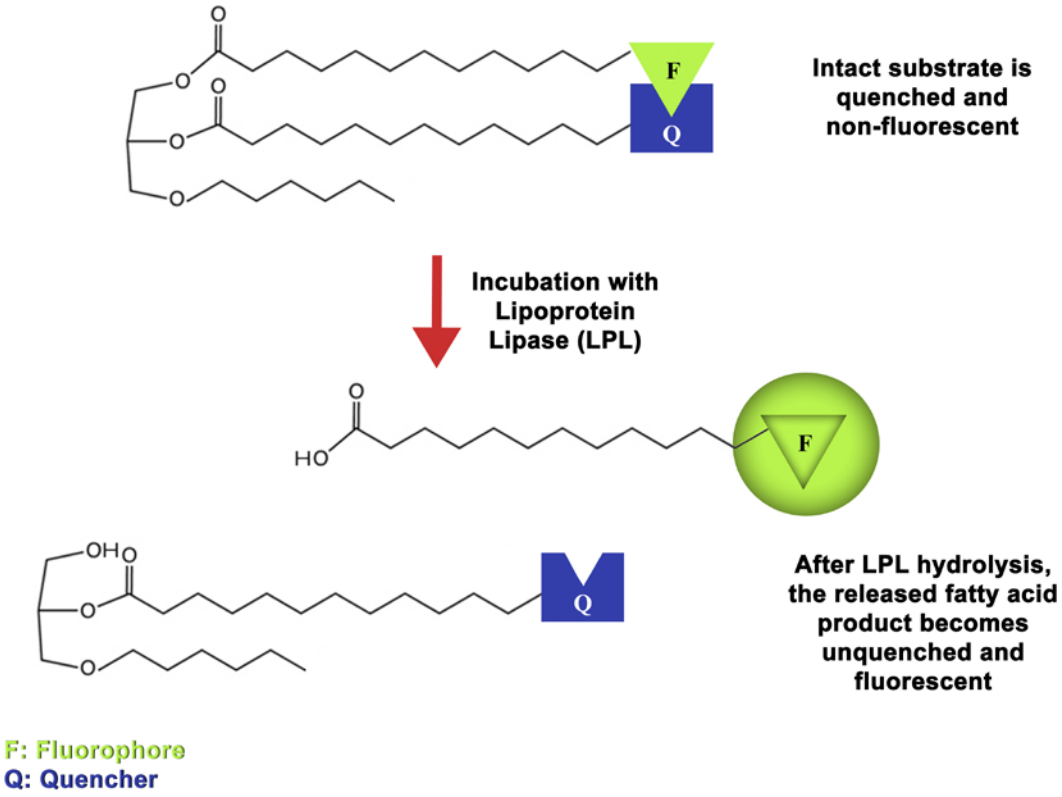
Cell Biolabs' Lipoprotein Lipase Activity Assay Kit utilizes a fluorogenic triglyceride analog as a lipase substrate. When uncleaved, the substrate remains in a non-fluorescent, quenched state; however, upon hydrolysis by LPL at the *sn*-1 position, a fluorescent product is produced which can be measured in a fluorescence microplate reader (Ex. 480-485 nm/Em. 515-525 nm with 495 nm cutoff) (See Assay Principle).

The Lipoprotein Lipase (LPL) Activity Assay Kit is a simple, fluorometric assay that quantitatively measures LPL activity in plasma, serum, and lysates in a 96-well microtiter plate format. Each Trial Size LPL Activity Assay Kit provides sufficient reagents to perform up to 20 assays, including blanks, LPL standards, and unknown samples. The kit contains a LPL Standard and has a detection sensitivity limit of ~1 mUnits/mL (see Kit Components for unit definition). Besides LPL, this assay can also be used to detect endothelial and hepatic lipase activity.

Related Products

1. STA-241: Human Low Density Lipoprotein
2. STA-242: Human Very Low Density Lipoprotein
3. STA-361: Human ApoAI and ApoB Duplex ELISA Kit
4. STA-368: Human ApoB ELISA Kit
5. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
6. STA-375: Uric Acid/Uricase Assay Kit
7. STA-378: Creatinine Assay Kit
8. STA-390: Total Cholesterol Assay Kit
9. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
10. STA-396: Serum Triglyceride Quantification Kit (Colorimetric)
11. STA-397: Serum Triglyceride Quantification Kit (Fluorometric)
12. STA-398: Free Glycerol Assay Kit (Colorimetric)
13. STA-399: Free Glycerol Assay Kit (Fluorometric)

Assay Principle



Kit Components

1. LPL Fluorometric Substrate (Part No. 261001-T): One 5 µL amber tube.
2. 10X LPL Assay Buffer (Part No. 261002): One 1.5 mL tube.

   Note: This buffer does not contain calcium or magnesium.
3. Stop Solution (Part No. 261003): One 1.5 mL tube.
4. LPL Enzyme Standard (Part No. 261004-T): One 5 µL tube provided at 2500 Units/mL.

   Note: One unit corresponds to the amount of enzyme which liberates 1 µmol oleic acid per minute at pH 8.0 and 40ºC, using triolein as a substrate.

Materials Not Supplied

1. Standard 96-well fluorescence black microtiter plate
2. 37ºC incubator
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. Fluorescence microplate reader equipped with an excitation filter at 480-485 nm and an emission filter at 515-525 nm (495 nm cutoff is recommended)

Storage

Store the entire kit at -80ºC. Avoid multiple freeze/thaws by aliquoting. The LPL Fluorometric Substrate is light sensitive and should be maintained in amber tubes.

Preparation of Reagents

- 1X LPL Assay Buffer: Dilute the 10X LPL Assay Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- LPL Fluorometric Substrate: Thaw the LPL Fluorometric Substrate at room temperature during assay preparation. Immediately before use, dilute the LPL Fluorometric Substrate 1:500 with 1X LPL Assay Buffer. Briefly vortex to homogeneity. Do not store diluted solutions.
  Note: For longer term storage, the LPL Fluorometric Substrate should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.
- Stop Solution: Thaw the Stop Solution at 37ºC. For longer term storage, the Stop Solution may be stored at room temperature or 4ºC. If crystals are present, briefly heat the solution at 37ºC to redissolve.

Preparation of LPL Standard Curve

- LPL Enzyme Standard should be thawed/maintained at 4ºC during assay preparation. For longer term storage, the LPL Enzyme Standard should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.
- To prepare the LPL enzyme standards, first perform a 1:100 dilution of the stock 2500 Units/mL LPL Enzyme Standard in 1X LPL Assay Buffer. Prepare only enough for immediate use (e.g. Add 2 µL of 2500 Units/mL LPL Enzyme Standard to 198 µL 1X LPL Assay Buffer). This solution has a concentration of 25 Units/mL. Use this 25 Units/mL solution to prepare standards in the concentration range of 0 – 250 mUnits/mL by further diluting in 1X LPL Assay Buffer (e.g. Add 5 µL of 25 Units/mL LPL solution to 495 µL 1X LPL Assay Buffer - see Table 1 below). Do not store diluted standards.

Standard Tubes	25 Units/mL LPL Enzyme Standard (µL)	1X LPL Assay Buffer (µL)	Final LPL Enzyme Standard (mUnits/mL)
1	5	495	250
2	250 of tube #1	250	125
3	250 of tube #2	250	62.5
4	250 of tube #3	250	31.3
5	250 of tube #4	250	15.6
6	250 of tube #5	250	7.8
7	250 of tube #6	250	3.9
8	0	250	0

Table 1. Preparation of LPL Enzyme Standard Curve

Preparation of Samples

- Plasma: Collect blood with an anticoagulant such as heparin, citrate or EDTA and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80°C for up to 1 month. Plasma must be diluted 1:50 to 1:200 in 1X LPL Assay Buffer before assaying.
- Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for up to 1 month. Serum must be diluted 1:50 to 1:200 in 1X LPL Assay Buffer before assaying.
- Cell Lysates: Collect 10 x 10^6 cells by centrifugation at 1000 x g for 10 minutes. Discard the supernatant and resuspend in 1 mL of cold, 20 mM Tris, pH 7.5, 150 mM NaCl. Homogenize or sonicate the cell suspension. Centrifuge at 10,000 x g for 10 minutes at 4°C. Carefully collect the supernatant and store on ice for immediate use. For longer term storage, freeze the lysate at -80°C for up to 1 month. Cell lysates must be further diluted 1:20 or greater in 1X LPL Assay Buffer before assaying.
- Tissue Samples: Weigh out 200 mg of tissue and mince into small pieces. Rinse the tissue with cold PBS to remove red blood cells and clots. Homogenize the minced tissue in 1 mL of cold, 20 mM Tris, pH 7.5, 150 mM NaCl. Centrifuge at 10,000 x g for 10 minutes at 4°C. Carefully collect the supernatant and store on ice for immediate use. For longer term storage, freeze the homogenate at -80°C for up to 1 month. Cell lysates must be further diluted 1:100 or greater in 1X LPL Assay Buffer before assaying.

Assay Protocol

Note: Maintain all LPL samples and standards at 4ºC during assay preparation.

1. Prepare and mix all reagents thoroughly before use.
2. Each LPL sample, LPL enzyme standard and blank should be assayed in duplicate.
3. Add 100 µL of the diluted LPL enzyme standards or samples to a 96-well fluorescence microtiter plate.
4. Add 100 µL of diluted LPL Fluorometric Substrate (see Preparation of Reagents Section) to each tested well. Mix thoroughly.
5. Cover the plate wells to protect the reaction from light.
6. Incubate for 30 minutes at 37ºC.
7. Stop the reaction by adding 20 µL of Stop Solution to each well.
8. Cover the plate wells to protect the reaction from light.
9. Incubate at room temperature for 15 minutes on an orbital shaker.

10. Read the plate with a fluorescence microplate reader equipped for excitation in the 480-485 nm range and for emission in the 515-525 nm range (495 nm cutoff is recommended).
11. Calculate the lipoprotein lipase activity within samples by comparing the sample fluorescence to the standard curve. Negative controls (without LPL) should be subtracted.

Example of Results

The following figure demonstrates typical LPL Activity Assay Kit results. Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Devices) with a 485/525 nm filter set and 495 nm cutoff. One should use the data below for reference only. This data should not be used to interpret actual results.

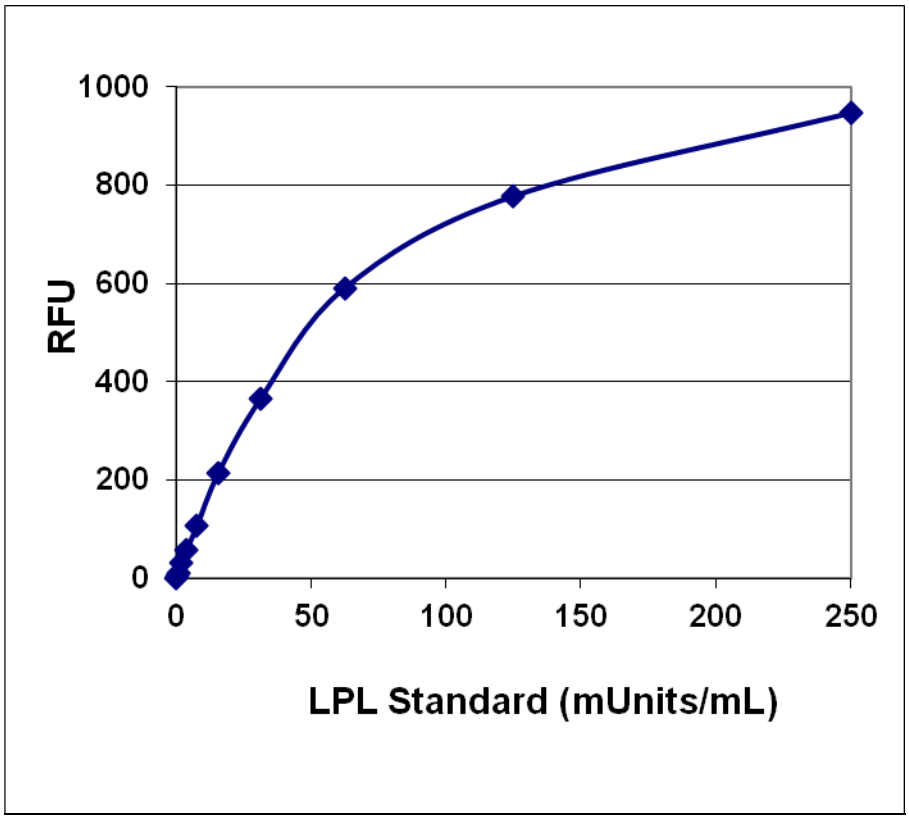


Figure 1: Lipoprotein Lipase Standard Curve.

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. Sithu, S.D. et al. (2017). Atherogenesis and metabolic dysregulation in LDL receptor–knockout rats. *JCI Insight* doi:10.1172/jci.insight.86442.
2. Mao, H., et al. (2017). Endothelial LRP1 regulates metabolic responses by acting as a co-activator of PPARγ. *Nat Commun.* **8**:14960. doi: 10.1038/ncomms14960.
3. An, Y.A., et al. (2017). Angiopoietin-2 in white adipose tissue improves metabolic homeostasis through enhanced angiogenesis. *Elife*. **6**. pii: e24071. doi: 10.7554/eLife.24071.
4. Aslan, İ. et al. (2016). Decreased eicosapentaenoic acid levels in acne vulgaris reveals the presence of a proinflammatory state. *Prostaglandins Other Lipid Mediat.* **128-129**:1-7. doi: 10.1016/j.prostaglandins.2016.12.001.
5. Liu, Z. et al. (2016). Extensive metabolic disorders are present in APC min tumorigenesis mice. *Mol Cell Endocrinol*. doi:10.1016/j.mce.2016.03.004.
6. Sun, X. et al. (2015). Insulin dissociates the effects of Liver X Receptor on lipogenesis, endoplasmic reticulum stress and inflammation. *J Biol Chem.* doi:10.1074/jbc.M115.668269.
7. Downing, L. E. et al. (2015). A grape seed procyanidin extract ameliorates fructose-induced hypertriglyceridemia in rats via enhanced fecal bile acid and cholesterol excretion and inhibition of hepatic lipogenesis. *PLoS One*. **10**:e0140267.
8. Brahma Naidu, P. et al. (2015). Ameliorative potential of gingerol: Promising modulation of inflammatory factors and lipid marker enzymes expressions in HFD induced obesity in rats. *Mol Cell Endocrinol.* doi:10.1016/j.mce.2015.10.007.
9. Qiao, L.et al. (2015). Maternal high fat feeding increases placenta lipoprotein lipase activity by reducing Sirt1 expression in mice. *Diabetes.* doi:10.2337/db14-1627.
10. Kim, H. K. et al. (2015). Regulation of energy balance by the hypothalamic lipoprotein lipase regulator Angptl3. *Diabetes*. **64**:1142-1153.Camporez, J. P. et al. (2015). ApoA5 knockdown improves whole-body insulin sensitivity in high-fat-fed mice by reducing ectopic lipid content. *J Lipid Res*. **56**:526-536.
11. Camporez, J. P. et al. (2015). ApoA5 knockdown improves whole-body insulin sensitivity in high-fat-fed mice by reducing ectopic lipid content. *J Lipid Res*. **56**:526-536.
12. Kim, H. Y. et al. (2015). Urine and serum metabolite profiling of rats fed a high-fat diet and the anti-obesity effects of caffeine consumption. *Molecules.* **20**:3107-3128.
13. Kassner, U. et al. (2015). Severe hypertriglyceridemia in a patient heterozygous for a lipoprotein lipase gene allele with two novel missense variants. *Eur J Hum Genet.* doi:10.1038/ejhg.2014.295.
14. Dib, L. et al. (2014). LXRα fuels fatty acid-stimulated oxygen consumption in white adipocytes. *J. Lipid. Res*. **55**:247-257.
15. Navab, M. et al. (2013). Transgenic 6F tomatoes act on the small intestine to prevent systemic inflammation and dyslipidemia caused by western diet and intestinally derived lysophosphatidic acid. *J. Lipid Res*. **54**:3403-3418.

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