



**KAMIYA BIOMEDICAL COMPANY**

# Lipoprotein Lipase (LPL) Activity Assay Kit (Fluorometric)

**For the quantitative determination of LPL activity in plasma, serum  
and lysates**

**Cat. No. KT-968**

**For Research Use Only. Not for use in diagnostic procedures.**

## Product Information

### Lipoprotein Lipase (LPL) Activity Assay Kit (Fluorometric) Cat. No. KT-968

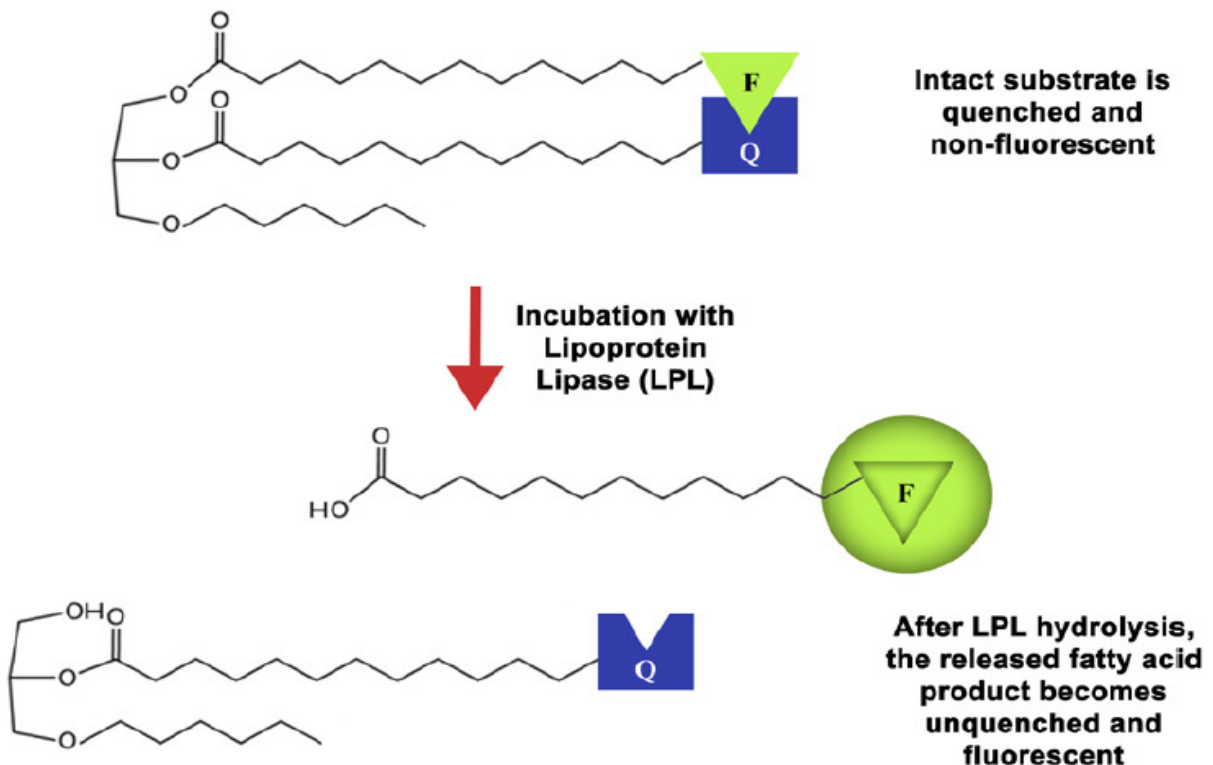
#### INTRODUCTION

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.

The 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 kit provides sufficient reagents to perform up to 100 assays, including blanks, LPL calibrators, and unknown samples. The kit contains a LPL Calibrator 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.

#### PRINCIPLE



**F: Fluorophore**  
**Q: Quencher**

## COMPONENTS

1. LPL Fluorometric Substrate: One 20  $\mu$ L amber tube.
2. 10X LPL Assay Buffer: Two 1.5 mL tubes. *Note: This buffer does not contain calcium or magnesium.*
3. Stop Solution: Two 1.5 mL tubes.
4. LPL Enzyme Calibrator

*Note: One unit corresponds to the amount of enzyme which liberates 1  $\mu$ mol oleic acid per minute at pH 8.0 and 40°C, using triolein as a substrate.*

## MATERIALS REQUIRED BUT NOT SUPPLIED

1. Calibrator 96-well fluorescence black microtiter plate
2. 37°C incubator
3. 10  $\mu$ L to 1,000  $\mu$ L adjustable single channel micropipettes with disposable tips
4. 50  $\mu$ L to 300  $\mu$ L 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 -80°C. Avoid multiple freeze/thaws by aliquoting. The LPL Fluorometric Substrate is light sensitive and should be maintained in amber tubes.

## REAGENT PREPARATION

- 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 -80°C to avoid multiple freeze/thaws.*
- Stop Solution: Thaw the Stop Solution at 37°C. For longer term storage, the Stop solution may be stored at room temperature or 4°C. If crystals are present, briefly heat the solution at 37°C to redissolve.

## CALIBRATOR PREPARATION

- LPL Enzyme Calibrator should be thawed/maintained at 4°C during assay preparation. For longer term storage, the LPL Enzyme Calibrator should be aliquoted and frozen at -80°C to avoid multiple freeze/thaws.
- To prepare the LPL enzyme calibrators, first perform a 1:100 dilution of the stock 2,500 Units/mL LPL Enzyme Calibrator in 1X LPL Assay Buffer. Prepare only enough for immediate use (e.g. Add 2  $\mu$ L of 2,500 Units/mL LPL Enzyme Calibrator to 198  $\mu$ L 1X LPL Assay Buffer). This solution has a concentration of 25 Units/mL. Use this 25 Units/mL solution to prepare calibrators in the concentration range of 0 – 250 mUnits/mL by further diluting in 1X LPL Assay Buffer (e.g. Add 5  $\mu$ L of 25 Units/mL LPL solution to 495  $\mu$ L 1X LPL Assay Buffer - see Table 1 below). Do not store diluted calibrators.

Calibrator Tubes	25 Units/mL LPL Enzyme Calibrator ( $\mu$ L)	1X LPL Assay Buffer ( $\mu$ L)	Final LPL Enzyme Calibrator (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 Calibration Curve**

## SAMPLE PREPARATION

- Plasma: Collect blood with an anticoagulant such as heparin, citrate or EDTA and mix by inversion. Centrifuge the blood at 1,000 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 2,500 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  $10 \times 10^6$  cells by centrifugation at 1,000 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 PROCEDURE

*Note: Maintain all LPL samples and calibrators at 4°C during assay preparation.*

1. Prepare and mix all reagents thoroughly before use.
2. Each LPL sample, LPL enzyme calibrator and blank should be assayed in duplicate.
3. Add 100  $\mu$ L of the diluted LPL enzyme calibrators or samples to a 96-well fluorescence microtiter plate.
4. Add 100  $\mu$ L 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 37°C.
7. Stop the reaction by adding 20  $\mu$ L 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 calibration 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.

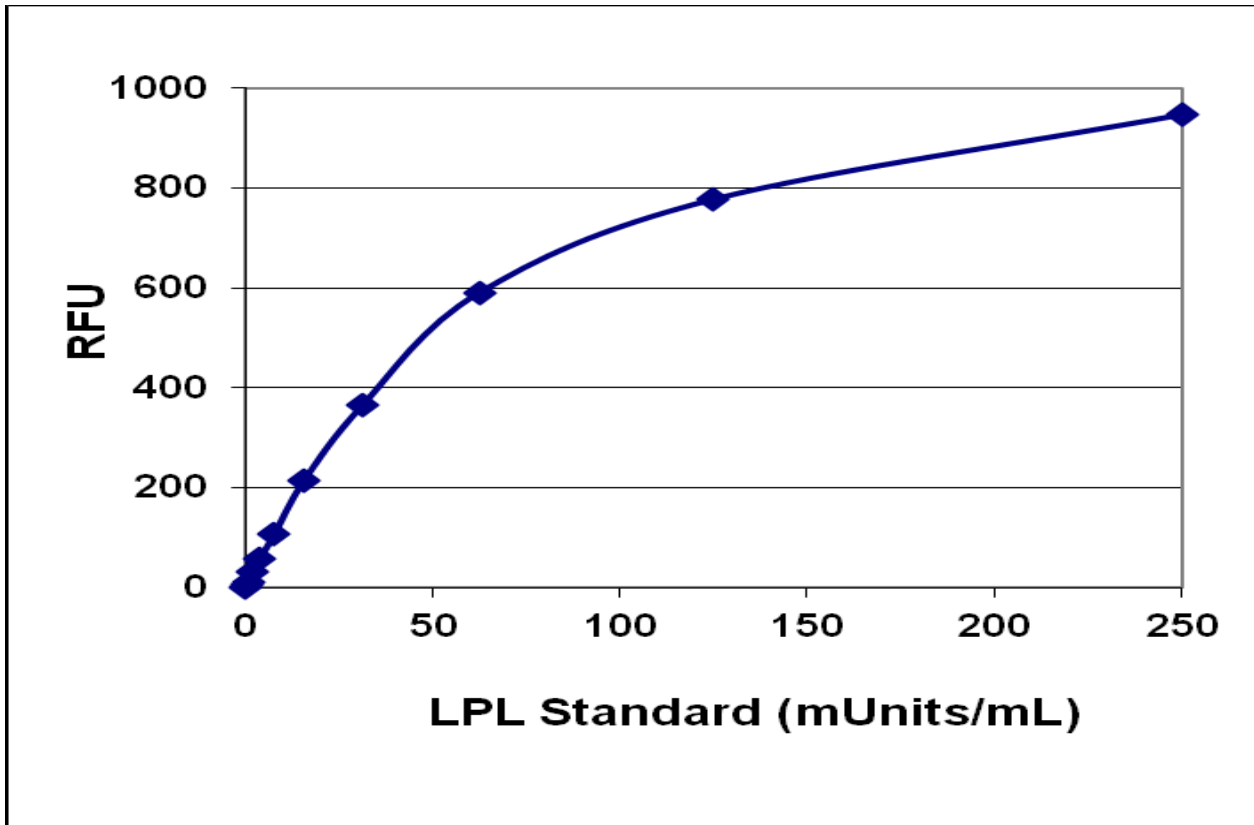


Figure 1: Lipoprotein Lipase Calibration Curve.

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