Lipoprotein Lipase Activity Assay Kit (Fluorometric)

9/14

(Catalog # K721-100; 100 assays; Store at 4°C)

I. Introduction:

Lipoprotein lipase (LPL) is a member of the lipase family that hydrolyzes triglycerides in chylomicrons and very low-density lipoprotein (VLDL). Digestion of triglycerides in VLDL by LPL leads to their conversion to intermediate-density lipoprotein (IDL) and then low-density lipoprotein (LDL). LPL is found attached to the luminal surface of endothelial cells in the heart, muscle, and adipose tissue. Mutations in lipoprotein lipase can lead to a variety of disorders such as lipoprotein metabolism, hypertriglyceridemia etc. Overexpression of LPL in mice has been shown to promote obesity and insulin resistance. BioVision's LPL Activity Assay Kit contains a quenched substrate that fluoresces upon hydrolysis by LPL. The fluorometric intensity is directly proportional to the amount of substrate hydrolyzed. This assay detects total lipase activity when no inhibitor is used. Comparing results in the presence or absence of an LPL inhibitor allows for quantification of LPL activity specifically. Our results indicate that the majority (~90%) of lipase activity detected by this kit in post-heparin treated mouse plasma is from LPL. To determine the exact LPL specific activity in mouse plasma, measure activity in pre- and post-heparin treated plasma.

II. Application:

- Measurement of LPL activity in purified wild type or recombinant protein as well as in plasma, cell and tissue lysates

III. Sample Type:

- Pure (wild type or recombinant) LPL protein
- Plasma
- Cell lysate and tissue (to determine LPL activity specifically compare results in the presence and absence of LPL inhibitor)

IV. Kit Contents:

Components	K721-100	Cap Code	Part Number
LPL Assay Buffer	5 ml	NM	K721-100-1
Substrate (in DMSO)	10 µl	Red	K721-100-2
Positive Control	Lyophilized	Blue	K721-100-3
Inhibitor (Orlistat)	20 µl	Yellow	K721-100-4

V. User Supplied Reagents and Equipment:

- White or black 96-well plate with flat bottom
- Multi-well fluorometer (fluorescence ELISA reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at 4°C protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. Upon opening, use kit within 2 months.

- **LPL Assay Buffer**: Warm to 37°C before use.
- **Substrate**: Dilute Substrate by adding 2 µl Substrate per 1.0 ml LPL Assay Buffer, mix well. Prepare as per assay requirement. Store unused Substrate at 4°C for up to 2 weeks. Warm to room temperature before reuse.
- **Positive Control**: Reconstitute with 220 µl of dH_2O to make stock solution. Make sure the material is completely dissolved. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Store at -20°C.
- **Inhibitor**: Ready to use as supplied. Warm to room temperature before use.

VII. LPL Activity Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissue (50 mg) or cells (10^6 cells) with 200 µl ice-cold PBS. Centrifuge at 10000 X g for 10 min. at 4°C. Collect supernatant. To measure maximum LPL activity in plasma, inject mouse/rat with 0.2 units heparin per g body weight by tail vein injection. Collect blood 10 min. after injection and spin at 3000 X g for 15 min. at 4°C. Collect supernatant into a clean tube. For recombinant or wild type purified enzyme, dissolve in dH_2O, PBS, or appropriate buffer. Add 1-10 µl plasma or enzyme, or 10-50 µl of homogenate into a 96-well plate. For Positive Control, dilute Positive Control stock 1:100 in dH_2O and add 4 µl of diluted Positive Control into desired well(s). Adjust the volume of Positive Control and samples to 50 µl/well with dH_2O. For the reagent background control, add 50 µl of dH_2O.
2. **Standard Curve Preparation:** Add 0, 2, 4, 6, 8, and 10 µl of diluted Substrate into a series of wells in a 96-well plate to give 0, 2, 4, 6, 8, and 10 pmol Substrate per well. Adjust the volume to 50 µl/well with LPL Assay Buffer.

   Notes:

   a. For unknown samples, do a pilot experiment, testing several amounts to ensure the readings are within the Standard Curve range.

   b. LPL is attached to endothelial cells by heparin-sulfated proteoglycans. Injecting heparin into mouse/rat releases LPL into the blood. Plasma can be isolated to measure the LPL activity.

   c. Optional: For assay validation, add 4 µl of diluted Positive Control and 2 µl of Inhibitor into desired well(s) and adjust the volume to 50 µl/well with dH_2O. Incubate at 37°C for 10 min.
3. **Reaction Mix**: For each Standard well, prepare 50 µl of Reaction Mix containing:

Positive Control	4 µl
dH_2O	46 µl

   Mix and add 50 µl of Reaction Mix into each Standard well.

To each sample, Positive Control and assay validation well, add 50 µl of diluted Substrate.

4. **Measurement:** Preincubate the plate at 37°C for 10 min. protected from light to stabilize the signal. Measure fluorescence (Ex/Em = 482/515 nm) kinetically for 10 min.-1 hr in a microplate reader at 37°C. Choose two points (T_1 and T_2) in the linear range of the plot and obtain the corresponding values (RFU_1 and RFU_2). Allow Standard to run for 30 min.
5. **Calculation:** Subtract 0 Standard reading from all Standard readings. Plot the Substrate Standard Curve. Subtract Background Control (BC) reading from Sample (S) reading.

$$\mathbf{\Delta RFU_S = RFU_{2S} - RFU_{1S}}$$
$$\mathbf{\Delta RFU_{BC} = RFU_{2BC} - RFU_{1BC}}$$
$$\mathbf{Corrected\ RFU = RFU_S - RFU_{BC}}$$

Apply sample's corrected RFU to Standard Curve to get B pmol of Substrate formed during the reaction time ΔT. Calculate sample's Lipoprotein Lipase activity by using the following equation:

$$\mathbf{Sample\ Lipoprotein\ Lipase\ Activity\ (A) = B/(\Delta T \times V)\ X\ D = pmol/ml/min = mU/ml}$$

Where: **B** is amount of substrate from Standard Curve (pmol)
V is sample volume added into the reaction well (ml)
ΔT is reaction time (min.)
D is sample Dilution factor

Alternatively, calculate the slope for all samples (S) and Background Control (BC) by dividing the net ΔRFU ($RFU_2 - RFU_1$) values of samples and background control by the time ΔT ($T_2 - T_1$). Subtract the slope of Background Control (BC) from the slope of sample to get the sample's corrected slope. To calculate LPL activity, divide sample's corrected slope by slope of Standard Curve (ΔRFU_C/pmol). The slope can also be obtained by plotting a graph (using a program such as Excel) and taking the m value of the y = mx + b equation. Use only the linear portion of the graph when obtaining the m value.

$$\mathbf{Lipoprotein\ Lipase\ activity\ (mU) = Slope_{Scorrected}/Slope_{Standard} = (\Delta RFU_s/\Delta T_s - \Delta RFU_{BC}/\Delta T_{BC})/(\Delta RFU_C/pmol)}$$

Where: $\mathbf{Slope_{Scorrected}}$ is the corrected slope of the sample
$\mathbf{Slope_{Standard}}$ is the slope of Standard Curve

Unit Definition: One unit of LPL is the amount of enzyme that generates 1.0 nmol of fatty acid product per min. at pH 7.4 at 37°C.

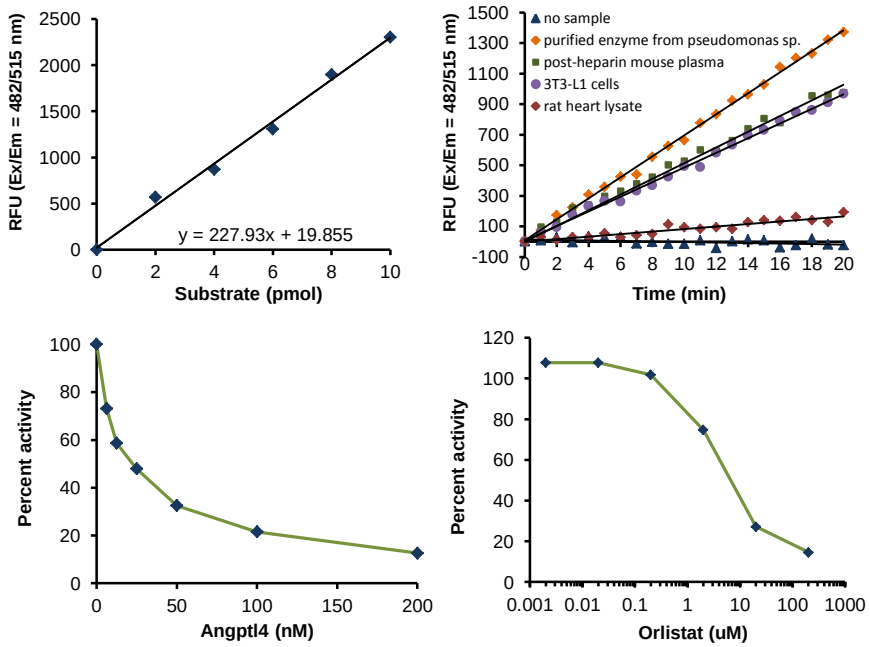

Figure: (a) Substrate Standard Curve, (b) Measurement of LPL activity in purified enzyme from pseudomonas sp. (5 ng), post-heparin treated mouse plasma (2 µl), 7-day post-differentiated 3T3-L1 cell lysate (100 µg), and rat heart lysate (200 µg). (c) Inhibition of LPL activity from post-heparin treated mouse plasma by Angptl 4, an LPL specific inhibitor. The assay was run for 1 hr and the activity was determined by calculating the slope. The IC_{50} was determined to be 22.6 nM. (d) Inhibition of Positive Control by Orlistat, a generic lipase inhibitor. The assay was run for 1 hr and the IC_{50} was determined to be 11.4 µM.

VIII. Related Products:

Lipase Activity Colorimetric Assay Kit (K722)
Lipase Activity Colorimetric Assay Kit II (K723)
Lipase Activity Fluorometric Assay Kit III (K724)
Phospholipase D (PLD) Activity Colorimetric Assay Kit (K725)
Lipoproteins, Human Plasma (4933, 4931, 4932)
HDL and LDL/VLDL Quantification Colorimetric/Fluorometric Kit (K613)
Triglyceride Quantification Colorimetric/Fluorometric Kit (K622)
PicoProbe™ Triglyceride (TG) Fluorometric Kit (K614)
LPL Blocking Peptide (3947BP)
LPL Antibody (3947)

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