Lipoprotein Lipase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1156

(Version 1.1A)

Detection and Quantification of Lipoprotein Lipase (LPL) Activity in Plasma, Cell Lysate, Tissue Lysate Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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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 lowdensity 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.

Lipoprotein Lipase Activity Colorimetric Microplate Assay Kit is a sensitive assay for determining Lipoprotein Lipase activity in various samples. The assay utilizes chromogenic substrate, which is hydrolyzed by LPL to release yellow product p-nitrophenol (p-NP). The color intensity increase at 410nm is proportional to the LPL activity in the sample. The assay detects total lipase activity when no inhibitor is used for majority (~90%) of lipase activity is from LPL. The assay also provides Orlistat as LPL specific inhibitor for assay validation.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	Powder x 2	4 °C, keep in dark
Substrate Diluent	1 ml x 1	4 °C
Activator	Powder x 1	4 °C
Emulsion Solution	20 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Standard Diluent	12 ml x 1	4 °C
Standard (0.4 mM)	Powder x 1	4 °C, keep in dark
Positive Control	Powder x 1	-20 °C
LPL Inhibitor (250X)	0.5 ml x 1	4 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 410 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Microcentrifuge tube
6. Centrifuge
7. Timer

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml Standard diluent to generate 10 mmol/L of standard stock solution, store at 4 °C for 1 day or -20°C for 3 days after reconstitution. Then dilute to 0.4 mmol/L standard top solution by adding 40 µl stock solution into 960 µl Standard diluent. Perform 2-fold serial dilutions of the top standard solution using Standard diluent to make the standard curve. The concentration of standard curve could be 0.4/0.2/0.1/0.05/0.025/0.0125/0.00625 mmol/L.

Substrate: Briefly centrifuge prior to opening. Dissolve each vial in 0.5 ml Substrate Diluent and heat to 50°C for 5-10 minutes to generate substrate stock solution. Keep in dark and store at room temperature or 4°C for 2-3 days.

Activator: Briefly centrifuge prior to opening. Add 0.6 ml distilled water to dissolve before use. Store at -20°C for 4-5 months after reconstitution.

Dye Reagent: Add 5 ml distilled water to dissolve before use. Store at 4 °C for 1 month after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Dissolve in 1 ml Assay Buffer before use. Store at -80 °C for 1 month.

LPL Inhibitor (1X): Briefly centrifuge LPL Inhibitor (250X) prior to opening. Then dilute to Inhibitor Working Solution by adding 4 µl stock solution into 996 µl distilled water.

Substrate Working Solution: Prepare Substrate Working Solution by mixing Substrate Solution, Activator and Emulsion Solution by vortexing. Keep in dark for immediate use The volumes of reagent could be enlarged according to the specified numbers of well. The table below gives some examples.

No. of wells	Substrate Solution (µl)	Activator (µl)	Emulsion Solution (µl)
1	5	5	130
10	50	50	1300
50	250	250	6500
100	500	500	13000

V. SAMPLE PREPARATION

1. For cell samples

Collect cell into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 cell (the quantity should be adjusted according to the actual situation of the sample), sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue (the quantity should be adjusted according to the actual situation of the sample), homogenize with 1 ml Assay Buffer on ice, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly or dilute with Assay Buffer.

VI. ASSAY PROCEDURE

Add following reagents in the microcentrifuge tube:

Reagent*	Sample**	Control	Positive Control	Inhibitor	Standard	Blank
Sample	10 µl	--	--	--	--	--
Positive Control	--	--	10 µl	10 µl	--	--
Distilled water	--	10 µl	--	--	--	150 µl
LPL Inhibitor (1X)	--	--	--	10 µl	--	--
Substrate Working Solution	140 µl	140 µl	140 µl	130 µl	--	--
Standard		--		--	150 µl	
Mix, incubate at 37°C for 5 minutes.						
Dye Reagent	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl
Mix, record absorbance measured at 410 nm.						

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of enzyme activity is defined as the enzyme hydrolyze 1 μmol of triglyceride per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample} - OD_{Control}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{Standard}}{V_{Sample}} \times n \quad (U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the quantity of cells

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay}) \times T} \quad (U/10^4)$$

2.2 According to the weight of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay}) \times T} \quad (U/g)$$

2.3 According to the volume of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T} \quad (U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of total sample, g

N: the quantity of total cell sample, 10^4

$C_{Standard}$: the concentration of standard, mmol/L = μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

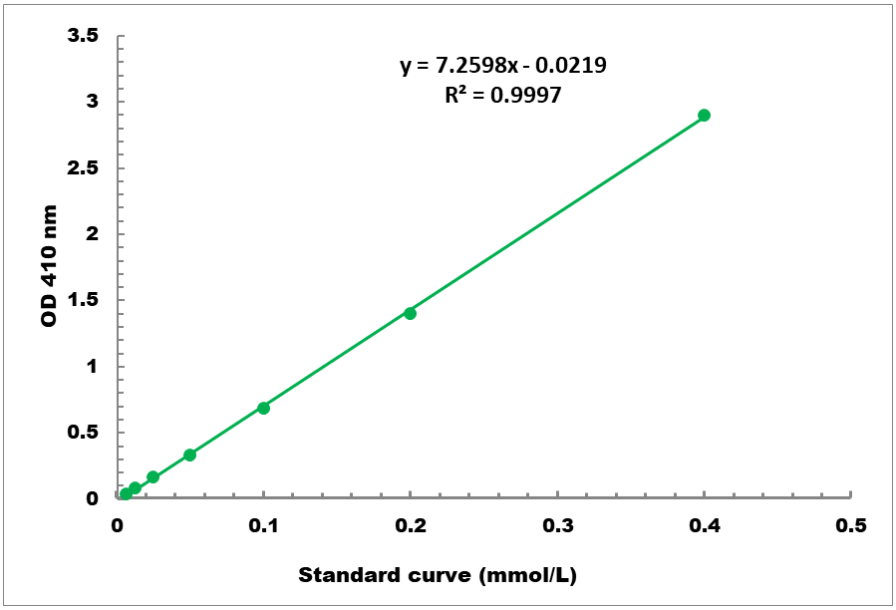
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

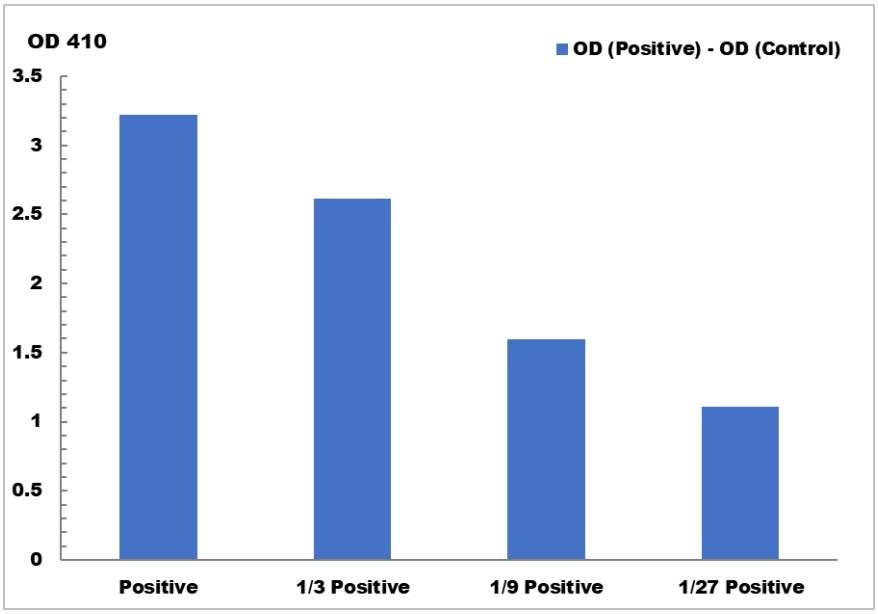
T: the reaction time, minute

VIII. TYPICAL DATA

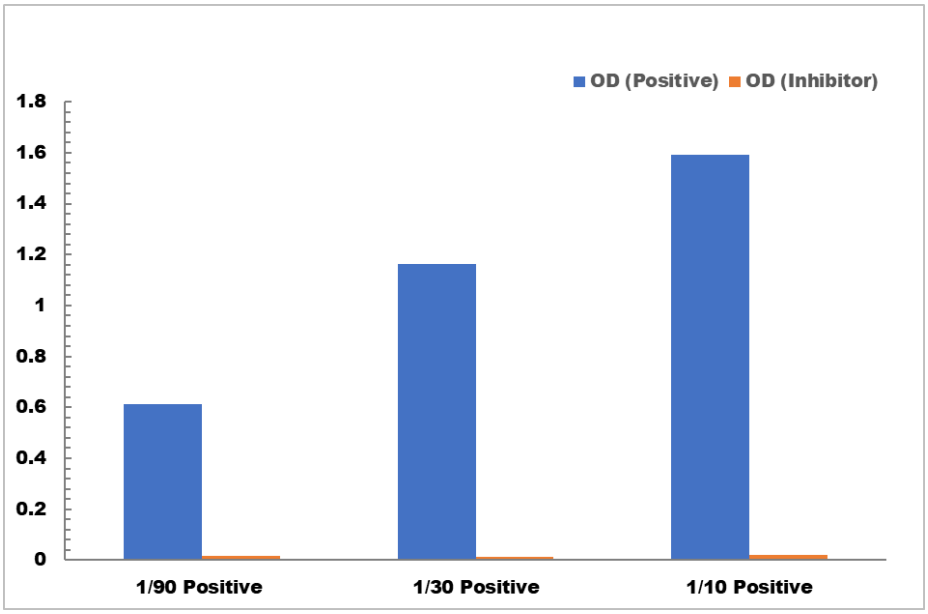
The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.004 mmol/L - 0.4 mmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration



Inhibitor reaction with decreasing Positive Control concentration