Lipase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1083

(Version 1.2C)

Detection and Quantification of Lipase (LPS) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

I. INTRODUCTION

Lipases perform essential roles in the digestion, transport and processing of dietary lipids (e.g. fats and oils) in living organisms. In humans, pancreatic lipase is the key enzyme responsible for breaking down fats in the digestive system by converting triglyceride to monoglyceride and free fatty acid. Pancreatic lipase monitoring is also used to help diagnose Crohn's disease, cystic fibrosis and celiac disease. Damage to the pancreas can exhibit a 5-10 fold increase of serum lipase levels within 24 to 48 hours.

The assay is initiated with the enzymatic catalysis of grease by LPS. The enzyme catalysed reaction products can be measured at a colorimetric readout at 710 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	10 ml x 1	4 °C
Reaction Buffer	10 ml x 1	4 °C
Extracting Solution	30 ml x 1	4 °C
Dye Reagent	10 ml x 1	4 °C, keep in dark
Standard (500 mmol/L)	1 ml x 1	4 °C
Technical Manual	1 Manual	

Note:

Substrate: Please mix and shake the substrate before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 710 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Vortex mixer

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 16,000g 4 °C for 40 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, homogenize with 1 ml Assay buffer on ice, centrifuged at 16,000g 4 °C for 40 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents in the microcentrifuge tube:

Reagent	Sample	Standard	Blank
Substrate	100 µl	--	100 µl
Reaction Buffer	100 µl	200 µl	100 µl
Mix and shake, vortex for 2 minutes.			
Sample	20 µl	--	--
Standard	--	20 µl	--
Distilled water	--	--	20 µl
Mix and shake, vortex for 2 minutes, put it in the oven, 40 °C for 30 minutes.			
Extracting Solution	300 µl	300 µl	300 µl
Mix and shake, vortex for 2 minutes, stay for 10 mintues, take the supernatant into a new microcentrifuge tube.			
The supernatant	200 µl	200 µl	200 µl
Dye Reagent	100 µl	100 µl	100 µl
Mix and shake, vortex for 2 minutes, stay for 5 mintues, take the supernatant into the microplate.			
The supernatant	100 µl	100 µl	100 µl
Record absorbance measured at 710 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of LPS activity is the enzyme that generates 1 μmol of Fatty acid per minute.

1. According to the protein concentration of sample

$$LPS\ (U/mg) = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 16.67 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$LPS\ (U/g) = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 16.67 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$LPS\ (U/10^4) = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 16.67 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of serum or plasma

$$LPS\ (U/ml) = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 16.67 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

$C_{Standard}$: the concentration of Standard, 500 μmol/ml;

$V_{Standard}$: the volume of standard, 0.02 ml;

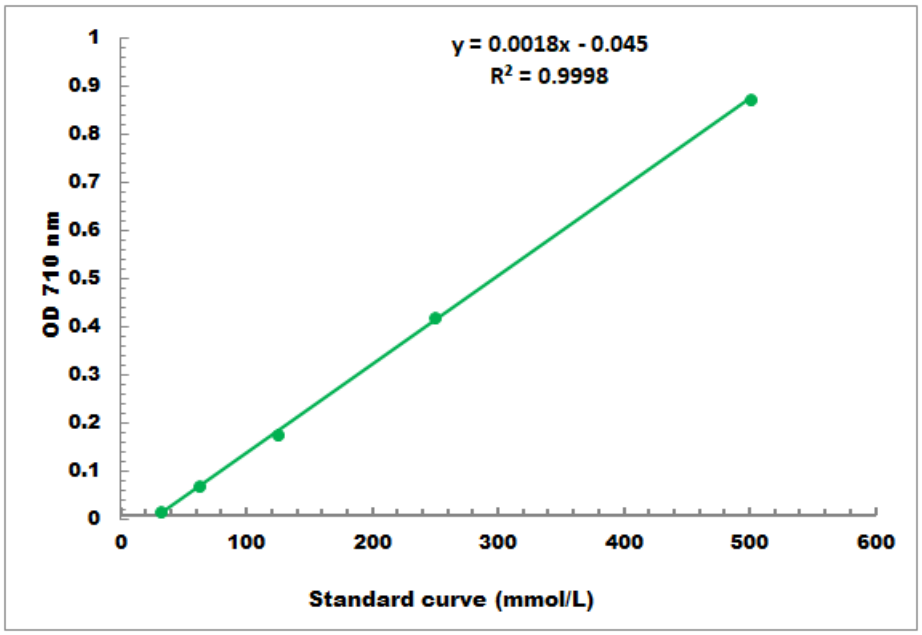
V_{Sample}: the volume of sample, 0.02 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 30 minutes.

VII. TYPICAL DATA

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



Detection Range: 50 mmol/L - 500 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES