Lipase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1083

(Version 1.3C)

Detection and Quantification ofLipase(LPS) Activityin Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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.

Lipase ActivityColorimetric Microplate Assay Kit provides a simple and direct procedure for measuring lipase activity in a variety of samples.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	30mlx 4	4 °C
Substrate	10 mlx 1	4 °C
Reaction Buffer	10 ml x 1	4 °C
Extracting Solution	30 mlx 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	

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. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 500/250/125/62.5/31.25mmol/L.

Substrate: Please mix and shake the substrate before use.

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay 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

Weighout 0.1 g tissue, homogenize with 1 mlAssay 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.

VI. ASSAY PROCEDURE

Warm allreagentsto 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 for2 minutes.			
Sample	20µl	--	--
Standard	--	20µl	--
Distilled water	--	--	20µl
Mix and shake, vortex for2 minutes, put it in the oven,40 °C for 30minutes.			
Extracting Solution	300 µl	300 µl	300 µl
Mix and shake, vortex for2 minutes, stay for 10 mintues, take the supernatant into anew microcentrifuge tube.			
The supernatant	200 µl	200 µl	200 µl
Dye Reagent	100µl	100µl	100µl
Mix and shake, vortex for2 minutes, stay for 5 mintues, take the supernatant into the microplate.			
The supernatant	100µl	100µl	100µl
Record absorbance measured at 710 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 LPSactivity is the enzyme thatgenerates 1μmol of Fatty acid per minute.

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

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

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

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

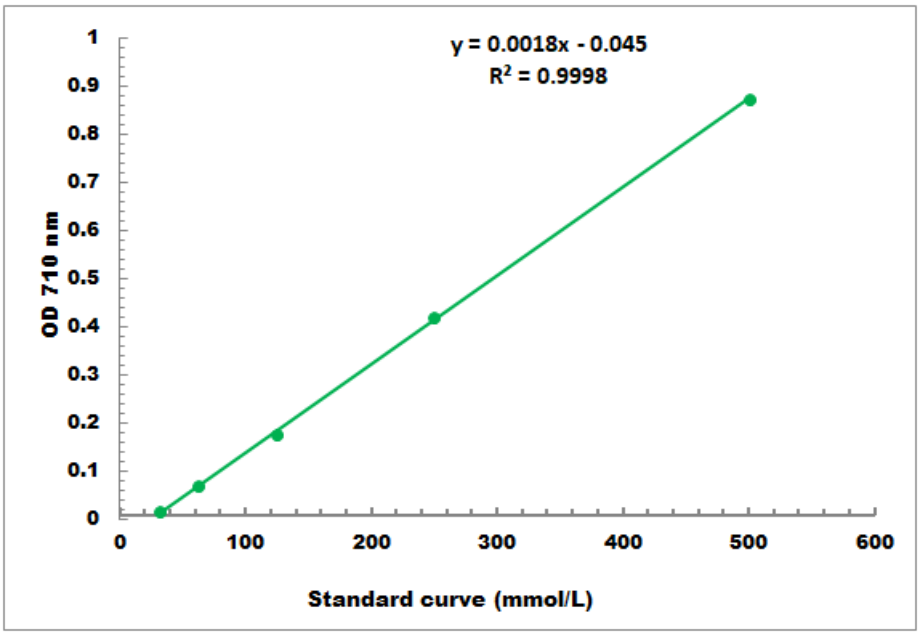
V_{Sample}: the volume of sample in assay procedure, mL

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: 50 mmol/L - 500 mmol/L