Lipase (LPS) Assay Kit

Note: Take two or three different samples for prediction before test.

Detection instrument: Spectrophotometer/ microplate reader

Cat No: BC2345

Size: 100T/96S

Components:

Reagent I: 120 mL×1. Store at 4℃.

Reagent II: 6 mL×1. Store at RT.

Reagent III: 10 mL×1. Store at 4℃.

Standard: 59.3 μL×1×1. Store at 4℃. 1.435 mL anhydrous ethanol is added to form 125 μmol/mL oleic acid standard solution, fully dissolved before use. Thawing completely before use.

Product Description:

LPS, also known as glyceride hydrolase, catalyzes the hydrolysis of triglycerides into fatty acids and glycerol (or diglycerides and monoesters). LPS is found in a wide variety of organisms. The abnormal increases of LPS in serum may indicate pancreatitis and pancreatic cancer.

LPS catalyzed the hydrolysis of oil esters into fatty acids. The formation rate of fatty acids was determined by copper soap method.

Required but mot provided

Mortar/homogenizer, centrifuge, pipette, spectrophotometer/ microplate reader, micro glass cuvette/ 96 well flat-bottom plate (non polystyrene material.), transferpettor, methylbenzene, anhydrous ethanol, ice and distilled water.

Procedure:

I. Sample Extraction:

1) Tissue sample:

Suggested 0.1 g tissue with 1 mL Reagent I. Fully grinding on ice. Centrifuge at 15000 rpm and 4℃ for 30 min, take the supernatant for testing.

2) Serum sample:

Detect sample directly.

II. Determination procedure:

1 Preheat spectrophotometer/ microplate reader for 30 min, adjust wavelength to 710 nm and set zero with methylbenzene.

2 Preheat Reagent I and Reagent II in 37℃ water bath for 30 min.

3 Dilution of standard solution: dilute the 125 μmol/mL oleic acid standard solution to 125, 62.5, 31.25, 15.625, 7.8125, 3.9 μmol/mL with anhydrous ethanol.

4 Add reagents with the following list:

Reagent (mL)	Blank control (B)	Test tube (T)	Standard tube (S)
Reagent I	0.15	0.15	0.15
Reagent II	0.05	0.05	0.05
Mix thoroughly			
Distilled water	0.08		
Supernatant or serum		0.08	
Standard solution			0.08
Vortex blending rapidly and then in 37℃ water bath for 10 min accurately			
Methylbenzene	0.4	0.4	0.4
Vortex blending repeatedly and then 4000 rpm centrifuge for 10 min			

Take out the tube and absorb 0.9 mL supernatant solution add to another new 2 mL tube, then add Reagent III as follow:

Reagent (mL)	Blank control (B)	Test tube (T)	Standard tube (S)
Reagent III	0.075	0.075	0.075

Vortex blending repeatedly, then centrifuge at 4000 rpm for 10 min at room temperature, take 200 μL supernatant solution carefully, add the solution to micro glass cuvette/ 96 well flat-bottom plate, measure the absorbance of each sample at 710 nm. $A_{blank\ tube}$=A(B), $A_{test\ tube}$=A(T), $A_{Standard\ tube}$ =A(S), ΔA (T)=A(T)-A(B), ΔA (S)=A(S)-A(B)

III. Calculation:

1 Drawing standard curve

Using the concentration of standard solution as x axis and ΔA(S) (ΔA=A(S)-A(B)) as y axis create standard curve, obtain equation y=kx+b. Put ΔA(T) into the equation and obtain the x (μmol/mL)

2 Enzyme activity calculation:

1) Calculated by protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the hydrolysis of olive oil to 1 μmol fatty in the reaction system per minute at 37℃ every mg protein.

LPS (U/mg prot) =x×Vs÷(Cpr×Vs) ÷T= 0.1×x÷Cpr

2) Calculated by sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the hydrolysis of olive oil to 1 μmol fatty in the reaction system per minute at 37℃ every g sample.

LPS (U/g fresh weight) =x×Vs÷(W×Vs÷Ve) ÷T= 0.1×x÷W

3) Calculated by serum:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the hydrolysis of olive oil to 1 μmol fatty in the reaction system per minute at 37℃ every mL serum.

LPS (U/mL serum) =x÷T= 0.1×x

Vs: supernatant volume in reaction system, 0.08 mL;

Cpr:sample extraction concentration, mg/mL; need to detect separately, suggest use PC0020, BCA Protein Assay Kit;
T: reaction time, 10 min;
W: Sample weight, g;
Ve: Extraction solution volume,1 mL.

Note:

1．methylbenzene is toxic, please ware gloves and masks during the experiment.
2．Keep away from fire during the experiment.
3．Suggest diluting the sample and measure again if the absorbance is greater than 1.
4．If the enzyme plate is used for the test, it is recommended to use 96 well flat-bottom plate made of non-polystyrene material.

Experimental example:

1. Take 0.1g pancreatic tissue, add 1ml reagent - homogenate, grind, take the supernatant, and then operate according to the determination steps. Calculate $\Delta A_T = A_T - A_B = 0.9734-0.1213 = 0.8521$, standard curve y = 0.0036x + 0.0123, then x = (0.8521-0.0123) ÷ 0.0036 = 233.278
LPS (U/g mass) = 0.1×x ÷ W = 0.1×233.278 ÷ 0.1 = 233.278 U/g mass.

Recent Product Citation：

[1] Jing Ge,Tao Han,Xiaoqiu Li,et al. S-adenosyl methionine regulates calcium channels and inhibits uterine smooth muscle contraction in rats with infectious premature delivery through the transient receptor protein 3/protein kinase Cβ/C-kinase-activated protein phosphatase-1 inhibitor of 17 kDa signaling pathway. Experimental and Therapeutic Medicine. July 2018;(IF1.410)

[2] Zhen X, Gao F, Li X, et al. Responses of hypocotyl growth and seedling emergence with respect to soil sowing depth stress in peanut (Arachis hypogaea L.)[J]. Archives of Agronomy and Soil Science, 2020: 1-17.

Related Products：

BC0590/BC0595 Free fatty Acids(FFA) Content Assay Kit
BC1080/BC1085 Alcohol Dehydrogenase(ADH) Activity Assay Kit
BC0320/BC0325 Plant Lipoxygenase(LOX) Activity Assay Kit