Lipase(LPS) Assay Kit

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

Detection instrument: Spectrophotometer

Cat No: BC2340

Size: 50T/48S

Components:

Reagent I: 80 mL×1. Store at 4℃.

Reagent II: 10 mL×1. Store at RT.

Reagent III: 20 mL×1. Store at 4℃.

Standard: 59.3 μL×1. Store at 4℃. 1.435 mL anhydrous ethanol was 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, 1 mL glass cuvette, 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 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	0.375	0.375	0.375
Reagent II	0.125	0.125	0.125
Mix thoroughly			
Distilled water	0.2		
Supernatant or serum		0.2	
Standard solution			0.2
Vortex blending rapidly and then in 37 ℃ water bath for 10 min accuratly			
methylbenzene	1	1	1
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	0.225	0.225	0.225

Vortex blending repeatedly, then centrifuge at 4000 rpm for 10 min at room temperature, take 800 μL supernatant solution carefully, add the solution to 1 mL glass cuvette, 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, 0.2 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.

Experimental example:

1. Take 0.1g pancreatic tissue, add 1 mL of Reagent I, homogenate, take the supernatant, and then operate according to the determination steps. Use 96 well plate to measure and calculate $\Delta A_T=A_T-A_B$= 0.811-0.112 = 0.699, standard curve y = 0.0074x, then x = 0.699 ÷ 0.0074 = 94.459.
   LPS (U/g mass) = 0.1×x ÷ W = 0.1×94.459 ÷ 0.1 = 94.459 U/g mass.
2. Take 0.1g Photinia rubra, add 1 mL of Reagent I, homogenate, take the supernatant, and then operate according to the determination steps. Use 96 well plate to measure and calculate $\Delta A_T=A_T-A_B$= 0.131-0.112 = 0.019, standard curve y = 0.0074x, then x = 0.019 ÷ 0.0074 = 2.568.
   LPS (U/g mass) = 0.1×x÷ W = 0.1×2.568 ÷ 0.1 = 2.568 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