Lipase (LPS) Activity Assay Kit

Note: The reagents have changed, please operate in strict accordance with the instructions.

Detection instrument: Spectrophotometer/ microplate reader

Cat No: BC2345

Size: 100T/96S

Components:

Reagent I: Liquid 125 mL×1. Store at 2-8℃.

Reagent II: Liquid 3 mL×1. Store at 2-8℃.

Reagent III: Powder×1. Store at 2-8℃. Add 12 mL distilled water to dissolve it in boiling water. It could be stored at 2-8℃ for two weeks.

Reagent IV: Liquid 10 mL×1. Store at 2-8℃.

Standard: Liquid 59.3 μL×1. Store at 2-8℃. Add 1.435 mL anhydrous ethanol to form 125 μmol/mL oleic acid standard solution, fully dissolved before use. Thawing completely before use. It could be stored at 2-8℃ for one month.

Working solution: Reagent II and Reagent III are mixed by the ratio of 1mL: 4mL（5mL, 50T）to make working solution according to sample number. Mix it at high speed on a vortex mixer (work time 3min, interval 5min, repeat once).

Product Description:

Lipase (LPS, EC 3.1.1.3), 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 not provided

Mortar/homogenizer, centrifuge, 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) Cell:

Collect bacteria or cells into the centrifuge tube, discard supernatant after centrifugation. According to the proportion of cells number (10^4): reagent I volume (mL) of 500-1000-1 to extract. It is suggested that 5 million of cell amount with 1mL of reagent I. Split cell with ultrasonication (placed on ice, ultrasonic power 200W, working time 3s, interval 10s, repeat for 30 times). Centrifuge at 15000 rpm for 15minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.

2) Tissue sample:

According to the ratio of tissue mass (g): reagent I volume (mL) to 1:5~10 (it is recommended to weigh about 0.1g of tissue, add 1mL of reagent I), and perform ice bath homogenization. 4℃, 15000rpm centrifugation for 15min, take the supernatant for testing.

3) Serum sample:

Detect sample directly. (If the liquid is turbid, measure after centrifugation)

Note: The supernatant of high-fat samples may have solid lipids after centrifugation. It should be removed by cotton stick before test.

II. Determination procedure:

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

2 Preheat reagent I and working solution in 37℃ water bath for 10 min.

3 Dilution of standard solution: dilute the 125 μmol/mL oleic acid standard solution to 31.25, 15.625, 7.8125, 3.9, 1.95, 0.975 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	0.1	0.1	0.1
Working solution	0.1	0.1	0.1
Distilled water	0.1	-	-
Sample	-	0.1	-
Standard solution	-	-	0.1
Vortex blending rapidly and then in 37 ℃ water bath for 20 min.			
methylbenzene	0.4	0.4	0.4
Vortex blending for 5min, then centrifuge at 8000 rpm for 10 min.			

Take out the tube and absorb 0.3 mL supernatant solution add to another 2 mL tube, then add Reagent IV as follow:

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

Repeated shaking for 3min, 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. $\Delta A_T=A_T-A_B$, $\Delta A_S=A_S-A_B$. Standard curve and blank tubes only need to be done 1-2 times.

III. Calculation:

1 Drawing standard curve

According to the concentration of the standard tube (x, μmol/mL) and the absorbance ΔA_S (y, ΔA_S), establish a standard curve. According to the standard curve, bring the ΔA_T (y, ΔA_T) into the formula to calculate the sample concentration (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 activity (U/mg prot) =x×Vs÷(Cpr×Vs) ÷T×F = 0.05×x÷Cpr×F

2) Calculated by cell number:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the hydrolysis of olive oil to 1 μmol fatty acid in the reaction system per minute at 37℃ every 10^4 cell.

LPS activty (U/10^4 cell) =x×Vs÷(N×Vs÷Ve) ÷T×F = 0.05×x÷N×F

3) 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 activity (U/g weight) =x×Vs÷(W×Vs÷Ve) ÷T×F = 0.05×x÷W×F

4) Calculated by serum volume:

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 activity (U/mL serum) =x÷T×F = 0.05×x×F

Vs: supernatant volume in reaction system, 0.1 mL;

Cpr: sample protein concentration, mg/mL; need to detect separately;

T: reaction time, 20 min;

W: sample weight, g;

Ve: added reagent I volume,1 mL;

N: cell number, count by 10^4;

F: dilution factor.

Note:

1. Methylbenzene is toxic, please wear gloves and masks during the experiment.
2. Keep away from fire during the experiment.
3. When the ΔA_T is greater than 1, it is recommended to dilute sample supernatant with reagent I. When the ΔA_T is smaller than 0.04, it is recommended to prolong reaction time at 37℃. When calculating, pay attention to modify the calculation formula synchronously.
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 0.1035g rat pancreatic tissue, add 1 mL of reagent I, homogenate, take the supernatant and dilute it ten times with reagent I. Then operate according to the determination steps. Measure and calculate $\Delta A_T=A_T-A_B$= 0.699-0.079 = 0.620, standard curve y = 0.0226x+0.0281, R^2=0.9991, then x = 26.190.

The enzyme activity is calculated according to the sample weight:

LPS activity (U/g weight) = 0.05×x÷W×F = 126.522 U/g weight.

2. Take 0.1021g peanut seed, add 1 mL of reagent I, homogenate, take the supernatant, and then operate according to the determination steps. Measure and calculate $\Delta A_T=A_T-A_B$= 0.141-0.079 = 0.062, standard curve y = 0.0226x+0.0281, R^2=0.9991, then x = 1.5. The enzyme activity is calculated according to the sample weight:

LPS activity (U/g weight) = 0.05×x÷W×F = 0.735 U/g weight.

Recent Product Citation:

[1] Ye S, Zhao L, Qi Y, Yang H, Hu Z, Hao N, Li Y, Tian X. Identification of azukisapogenol triterpenoid saponins from Oxytropis hirta Bunge and their aphicidal activities against pea aphid Acyrthosiphon pisum Harris. Pest Manag Sci. 2023 Jan;79(1):55-67. doi: 10.1002/ps.7172. Epub 2022 Sep 26. PMID: 36067067.

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[3] Qian W, Guo M, Peng J, Zhao T, Li Z, Yang Y, Li H, Zhang X, King-Jones K, Cheng D. Decapentaplegic retards lipolysis during metamorphosis in Bombyx mori and Drosophila melanogaster. Insect Biochem Mol Biol. 2023 Apr; 155:103928. doi: 10.1016/j.ibmb.2023.103928. Epub 2023 Mar 2. PMID: 36870515.

[4] Cheng S, Li B, Ding Y, Hou B, Hung W, He J, Jiang Y, Zhang Y, Man C. The probiotic fermented milk of Lacticaseibacillus paracasei JY062 and Lactobacillus gasseri JM1 alleviates constipation via improving gastrointestinal motility and gut microbiota. J Dairy Sci. 2023 Nov 1: S0022-0302(23)00777-4. doi: 10.3168/jds.2023-24154.

[5] Chen Y, Tao H, Chen R, Pan Y, Wang J, Gao R, Chen J, Yang J. Biomimetic Nanoparticles Loaded with Ulinastatin for the Targeted Treatment of Acute Pancreatitis. Mol Pharm. 2023 Aug 7;20(8):4108-4119. doi: 10.1021/acs. molpharmaceut.3c00238. Epub 2023 Jun 22. PMID: 37349264.

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BC1080/BC1085	Alcohol Dehydrogenase (ADH) Activity Assay Kit
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