L-Lactate Dehydrogenase (L-LDH) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC0680

Size:50T/24S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent Name	Size	Preservation Condition
Extract solution	Liquid 30 mL×1	2-8℃
Reagent I	Liquid 25 mL×1	2-8℃
Reagent II	Powder ×1	-20℃
Reagent III	Liquid 25 mL×1	2-8℃
Reagent IV	Liquid 60 mL×1	2-8℃
Standard	Liquid 1 mL×1	2-8℃

Solution Preparation:

1. **Reagent II:** powder ×1 bottle, add 1.3 mL of distilled water before use. It can be divided into tubule after matching, the unused reagent can be stored at -20℃ for 2 weeks, avoid repeated freezing and thawing.
2. **Standard:** liquid ×1 bottle, 20 μmol/mL Sodium pyruvate.

Product Description:

L-Lactate dehydrogenase (L-LDH or LD) is the terminal enzyme of the glycolysis pathway which is found in nearly all living cells (animals, plants, and prokaryotes). L-LDH catalyzes the conversion of lactate to pyruvic acid and back, as it converts NAD^+ to NADH and back.

NAD^+ and lactic acid is oxidized to pyruvic acid by the catalysis of L-LDH. Pyruvate further reacted with 2,4-dinitrophenylhydrazide to form pyruvate dinitrobenzone, which show brown red color in alkaline solution and the color depth is proportional to the concentration of pyruvate.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, thermostat water bath, desk centrifuge, adjustable pipette, 1 mL glass cuvette, mortar/homogenizer/cell ultrasonic crusher, ice, distilled water.

Procedure:

I. Sample Preparation.(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1.Bacteria or cells

Collecting bacteria or cells into the centrifuge tube. The liquid in the upper layer was discarded after centrifugation. The ratio of bacteria/cell amount (10^4): Extract solution volume(mL) is 500~1000: 1 (it is

suggested to take about 5 million bacteria/cell and add 1 mL of Extract solution). Bacteria and cell is split
by ultrasonic (placed on ice, 200W, work time 3s, interval 10s, repeat for 30 times). Centrifuge at 8000 rpm 4℃ for 10 minutes, take the supernatant and put it on ice for testing.

2.Tissue

Ice-bath homogenate was conducted according to the ratio of tissue mass (g): Extract solution volume (mL) = 1: 5~10 (it is suggested to take about 0.1 g tissue and add 1 mL of Extract solution). Centrifuge at 8000 rpm 4℃ for 10 minutes, take the supernatant and put it on ice for testing.

3.Serum (plasma) sample:

Detect sample directly.

Note: The sample homogenate supernatant of the kit is also available for BC0090/BC0095 (Peroxidase), BC0020/BC0025 (Malondialdehyde), BC0200/BC0205 (Catalase), BC0170/BC0175 (Superoxide dismutase), and BC5160/BC5165 (superoxide dismutase).

II. Determination procedure.

1. Preheat the spectrophotometer 30 minutes, adjust wavelength to 450 nm, set zero with distilled water.
2. Sodium pyruvate Standard Solution:

20 μmol/mL standard solution is respectively diluted to 2, 1, 0.5, 0.25, 0.125 and 0 mmol/mL with distilled water. The standard curve is made through 2, 1, 0. 5, 0. 25, 0.125, 0 mmol/mL standard solution.

3. Sample Test

Reagent name (μL)	Test tube (T)	Control tube (C)	Standard tube (S)
Sample	50	50	-
Standard Solution	-	-	50
Reagent I	250	250	250
Reagent II	50	-	-
Distilled water	-	50	50
Mixed thoroughly, incubate at 37℃(mammal) or 25℃(other species) water bath for 15 minutes.			
Reagent III	250	250	250
Mixed thoroughly, incubate at 37℃(mammal) or 25℃(other species) water bath for 15 miutesn.			
Reagent IV	750	750	750

Mixed thoroughly, place at room temperature for 3 minutes, detect absorption at 450 nm, ΔA=At-Ac. Each test tube should set a control tube. (A_T: Test tube, A_C: Control tube)

III. L-LDH Calculations

1. Sample Sodium pyruvate content

Set the standard curve, x-axis as the standard concentration, μmol/mL; y-axis as the 450 nm absorption.Put ΔA(y) into standard curve, calculate x (μmol/mL)

2. **Serum (plasma) sample L-LDH activity**

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the producing of 1 nmol of pyruvic acid per minute every milliliter of serum.

L-LDH(U/mL)=x×Vs÷Vs÷T×10^3=66.7×x

3. **Tissue, bacteria or cultured cells L-LDH activity**

A. Calculate by sample protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the producing of 1 nmol of pyruvic acid per minute every milligram of protein.

L-LDH(U/mg prot)= x×Vs÷（Cpr×Vs）÷T×10^3=66.7×x÷Cpr

B. Calculate by sample mass

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the producing of 1 nmol of pyruvic acid per minute every gram of tissue.

L-LDH(U/g mass)= x×Vs÷（W÷Vsv×Vs）÷T×10^3=66.7×x÷W

C. Calculate by the number of bacteria or cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the producing of 1 nmol of pyruvic acid per minute every 1 0000 cells.

L-LDH (U/10^4 cell)= x×Vs÷（500÷Vsv×Vs）÷T×10^3=0.133×x

Vs: Supernatant volume (mL), 0.05 mL;
Vsv: Extract solution volume, 1 mL;
T: Reaction time, 15 minutes;
Cpr: Sample protein concentration, mg/mL;
W: Sample weight, g;
500: Total number of bacteria or cells, 5 million;
10^3: 1 μmol/mL=10^3 nmol/mL.

Note: When ΔA is greater than 1.3 or less than 0.01, it is recommended to dilute the sample with distilled water or increase the sample size for the experiment, and pay attention to the simultaneous modification of the calculation formula.

Experimental example:

1. Take 0.103 g of seduma leaves, add 1mL of extract solution, make ice bath homogenate, 8000g, centrifuge at 4℃ for 10min, take superclear ice to be measured. Then, according to the measurement procedure, 1 mL glass cuvette was used to calculate ΔA=A measuring tube -A control =0.206-0.134=0.072,

and the standard curve y=0.6238x+0.0227, R^2=0.9983, x=0.079 μmol/mL, and the lactate dehydrogenase activity was calculated to obtain:

L-LDH (U/g mass) =66.67×x÷W =51.14 U/g

2. Take 0.109 g of rabbit liver, add 1mL of extract liquid, homogenize in ice bath, 8000g, centrifuge at 4℃ for 10min, take supernatant and dilute 80 times with distilled water, then place on ice to be measured.

Then, according to the measurement procedure, 1 mL glass cuvette was used to calculate ΔA=A

measuring tube -A control = 0.936-0.095 =0.841, and the standard curve y=0.6238x+0.0227, R^2=0.9983, x= 1.312 μmol/mL, and the lactate dehydrogenase activity was calculated to obtain:

L-LDH (U/g mass) =66.67×x÷W×dilution ratio =64198.93 U/g

3. After taking 50μL equine serum, follow the measurement procedure, using 1 mL glass cuvette to calculate ΔA=A measuring tube -A control =0.529-0.155=0.374, and adding the standard curve y=0.6238x+0.0227, R^2=0.9983, x=0.563 μmol/mL, and calculate the lactate dehydrogenase activity to obtain:

L-LDH (U/mL) =66.67 x x =37.535 U/mL

Recent Product Citations:

[1] Wu L, Chen F, Chang X, Li L, Yin X, Li C, Wang F, Li C, Xu Q, Zhuang H, Gu N, Hua ZC. Combined Cellular Thermometry Reveals That Salmonella typhimurium Warms Macrophages by Inducing a Pyroptosis-like Phenotype. J Am Chem Soc. 2022 Oct 26;144(42):19396-19409. doi: 10.1021/jacs.2c07287. Epub 2022 Oct 13. PMID: 36228296.

[2] Zheng X, Wang Q, Zhou Y, Zhang D, Geng Y, Hu W, Wu C, Shi Y, Jiang J. N-acetyltransferase 10 promotes colon cancer progression by inhibiting ferroptosis through N4-acetylation and stabilization of ferroptosis suppressor protein 1 (FSP1) mRNA. Cancer Commun (Lond). 2022 Dec;42(12):1347-1366. doi: 10.1002/cac2.12363. Epub 2022 Oct 8. PMID: 36209353; PMCID: PMC9759759.

[3] Jiang Y, Cao S, Zhou B, Cao Q, Xu M, Sun T, Zhao X, Zhou Z, Wang Y. Hemocytes in blue mussel Mytilus edulis adopt different energy supply modes to cope with different BDE-47 exposures. Sci Total Environ. 2023 Aug 10;885:163766. doi: 10.1016/j.scitotenv.2023.163766. Epub 2023 May 3. PMID: 37146804.

[4] Li L, Li F, Bai X, Jia H, Wang C, Li P, Zhang Q, Guan S, Peng R, Zhang S, Dong JF, Zhang J, Xu X. Circulating extracellular vesicles from patients with traumatic brain injury induce cerebrovascular endothelial dysfunction. Pharmacol Res. 2023 Jun;192:106791. doi: 10.1016/j.phrs.2023.106791. Epub 2023 May 6. PMID: 37156450.

[5] Zihan S, Lu L, Tao W, Bolin Z, Hongfei Z. Starch nanoparticles as a new ice crystal nucleator in Lactobacillus bulgaricus CICC 6097 cryoprotection. Int J Biol Macromol. 2023 Aug 17;251:126395. doi: 10.1016/j.ijbiomac.2023.126395. Epub ahead of print. PMID: 37595719.

References:

[1] Huang P H, Fu L C, Huang C S, et al. The uptake of oligogalacturonide and its effect on growth inhibition, lactate dehydrogenase activity and galactin-3 release of human cancer cells[J]. Food chemistry, 2012, 132(4): 1987-1995.

[2] Papaneophytou C, Zervou ME, Theofanous A. Optimization of a Colorimetric Assay to

Determine Lactate Dehydrogenase B Activity Using Design of Experiments[J]. Journal of Biomolecular Screening, 2021, 26(3): 383-399.

Related Products:

BC0740/BC0745	Hexokinase(HK) Activity Assay Kit
BC0540/BC0545	Pyruvate Kinase(PK) Activity Assay Kit
BC2250/BC2255	Phosphoglycerate Kinase(PGK) Activity Assay Kit
BC2270/BC2275	Fructose-bisphosphate aldolase(FBA) Activity Assay Kit

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