

Lactate Dehydrogenase (LDH) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: NA0815

Size:50T/24S

Components:

Extract solution: 30 mL×1. Storage at 4°C;

Reagent I: 25 mL×1. Storage at 4°C.

Reagent II: Powder×1. Storage at -20°C. Working solution: dissolve with 1.3 mL of distilled water before use. Avoid freeze and thaw cycles.

Reagent III: 25 mL×1. Storage at 4°C.

Reagent IV: 60 mL×1. Storage at 4°C.

Sodium pyruvate standard solution: 1 mL (2 μmol/mL) ×1. Storage at 4°C.

Product Description:

Lactate dehydrogenase (LDH or LD) is the terminal enzyme of the glycolysis pathway which is found in nearly all living cells (animals, plants, and prokaryotes). 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 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 cuvette, mortar/homogenizer, ice, distilled water.

Procedure:

I. Sample preparation:

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°C 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°C for 10 minutes, take the supernatant and put it on ice for testing.

3. Serum (plasma) sample:

Detect sample directly.

III. Procedure:

1. Preheat the spectrophotometer 30 minutes, adjust wavelength to 450 nm, set zero with distilled water.

2. Sodium pyruvate Standard Solution:

Take 100 μL of 2 $\mu\text{mol}/\text{mL}$ standard solution is respectively diluted to 1, 0.5, 0.25, 0.125 and 0 mmol/mL by double dilution. 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°C(mammal) or 25°C(other species) water bath for 15 minutes.			
Reagent III	250	250	250
Mixed thoroughly, incubate at 37°C(mammal) or 25°C(other species) water bath for 15 minutes.			
Reagent IV	750	750	750

Mixed thoroughly, place at room temperature for 3 minutes, detect absorption at 450 nm, $\Delta A = A_t - A_c$. Each test tube should set a control tube. (A_T : Test tube, A_C : Control tube)

Set the standard curve, y-axis as the standard concentration, $\mu\text{mol}/\text{mL}$; x-axis as the 450 nm absorption.

II. LDH Calculations

1. Sample Sodium pyruvate content

Put $\Delta A(x)$ into standard curve, calculate y ($\mu\text{mol}/\text{mL}$)

2. Serum (plasma) sample 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.

$$\text{LDH}(\text{U}/\text{mL}) = y \div T \times 10^3 = 66.7 \times y$$

3. Tissue, bacteria or cultured cells LDH activity

A. 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.

$$\text{LDH}(\text{U}/\text{mg prot}) = y \div T \div \text{Cpr} \times 10^3 = 66.7 \times y \div \text{Cpr}$$

B. Sample weight

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.

$$\text{LDH}(\text{U}/\text{g}) = y \div T \div W \times 10^3 = 666.7 \times y$$

C. Cell amount

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.

$$\text{LDH(U/10}^4 \text{ cell)} = y \div T \div 500 \times 10^3 = 0.133 \times y$$

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;

10³: 1 μmol/mL=10³ nmol/mL.

Recent Product Citations:

[1] Zhou F, Du J, Wang J. Albendazole inhibits HIF-1α-dependent glycolysis and VEGF expression in non-small cell lung cancer cells[J]. Molecular and cellular biochemistry, 2017, 428(1-2): 171-178.

[2] Zhang H, Da Z, Feng Y, et al. Enhancing the electricity generation and sludge reduction of sludge microbial fuel cell with graphene oxide and reduced graphene oxide[J]. Journal of cleaner production, 2018, 186: 104-112.

[3] Zhao B, Sun L, Jiang X, et al. Genipin protects against cerebral ischemia-reperfusion injury by regulating the UCP2-SIRT3 signaling pathway[J]. European journal of pharmacology, 2019, 845: 56-64.

[4] Zhao H L, Wu B Q, Luo Y, et al. Exogenous hydrogen sulfide ameliorates high glucose-induced myocardial injury & inflammation via the C1RP-MAPK signaling pathway in H9c2 cardiac cells[J]. Life sciences, 2018, 208: 315-324.

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.

Related Products:

NA0809/NA0567 Hexokinase(HK) Activity Assay Kit

NA0826/NA0584 Pyruvate Kinase(PK) Activity Assay Kit

NA0710/NA0469 Phosphoglycerate Kinase(PGK) Activity Assay Kit

NA0708/NA0467 Fructose-bisphosphate aldolase(FBA) Activity Assay Kit