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Ver.240829

Lactate Dehydrogenase (LDH) Assay Kit

BC6603-02 (100 Test/ 48 Samples)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

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 are 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.

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment. If the absorption value of the sample is not within the measurement range, it is recommended to dilute or increase the sample size for detection.

Kit components

Components	Volume	Storage
Extract Solution	60 mL×1	2-8°C
Reagent I	7 mL×1	2-8°C
Reagent II	Powder×1	-20°C
Reagent III	7 mL×1	2-8°C
Reagent IV	25 mL×1	2-8°C
Standard	1 mL ×1	2-8°C

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°C for 2 weeks, avoid repeated freezing a thawing.
2. **Standard:** liquid ×1 bottle, 20 $\mu\text{mol/mL}$ Sodium pyruvate.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, thermostat water bath, desk centrifuge, adjustable pipette, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, ice, distilled water.

Operation Procedures

Sample Preparation

1. Bacteria, cells or tissue sample

Collecting bacteria or cells into the centrifuge tube. The liquid in the upper layer is 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 3 second, interval 10 second, repeat for 30 times). Centrifuge at 8000 rpm 4°C for 10 minutes, take the supernatant and put it on ice for test.

2. Tissue

Ice-bath homogenate is 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 of tissue and add 1 mL of Extract solution). Ice bath homogenization. Centrifuge at 8000 rpm and 4°C for 10 minutes, take the supernatant and put it on ice for test.

3. Serum or Plasma

Detect sample directly

Procedure

1. Preheat the Spectrophotometer/Microplate reader 30 minutes, adjust wavelength to 450 nm, set zero with distilled water.
2. 20 $\mu\text{mol/mL}$ standard solution is respectively diluted to 2, 1, 0.5, 0.25, 0.125 and 0 mmol/mL with distilled water and use 2, 1, 0.5, 0.25, 0.125, 0 $\mu\text{mol/mL}$ as standard curve.
3. Sample Test

Reagent name(μL)	Test Tube (A_t)	Control Tube (A_c)	Standard Tube (A_s)
Sample	10	10	-
Standard Solution	-	-	10
Reagent I	50	50	50
Reagent II	10	-	-
Distilled water	-	10	10
Mixed thoroughly, incubate at 37°C (mammal) or 25°C (other species) water bath for 15 minutes.			
Reagent III	50	50	50
Mixed thoroughly, incubate at 37°C (mammal) or 25°C (other species) water bath for 15 minutes.			
Reagent IV	150	150	150

Mix thoroughly, place at room temperature for 3 minutes. Take 200 μL of reaction solution in micro glass cuvette/96 well flat-bottom plate, measured the absorbance at 450 nm, $\Delta A = A_t - A_c$. Each test tube should set a control tube.

Calculations

1. Set the standard curve, x-axis as the standard concentration, $\mu\text{mol/mL}$, y-axis as the 450 nm absorption. Put $\Delta A(y)$ into standard curve, calculate x ($\mu\text{mol/mL}$)
2. **Sample Sodium pyruvate content**
Put $\Delta A(y)$ into standard curve, calculate x ($\mu\text{mol/mL}$)
3. **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.

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

4. Tissue, bacteria or cultured cells 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.

$$\begin{aligned}\text{LDH(U/mg prot)} &= x \times V_s \div (C_{pr} \times V_s) \div T \times 10^3 \\ &= 66.7 \times x \div C_{pr}\end{aligned}$$

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.

$$\begin{aligned}\text{LDH(U/g)} &= x \times V_s \div (W \div V_{sv} \times V_s) \div T \times 10^3 \\ &= 66.7 \times x \div W\end{aligned}$$

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 10000 cells.

$$\begin{aligned}\text{LDH (U/10}^4 \text{ cell)} &= x \times V_s \div (N \div V_{sv} \times V_s) \div T \times 10^3 \\ &= 66.7 \times x \div N\end{aligned}$$

V_s: Supernatant volume (mL), 10 μL = 0.01 mL

V_{sv}: Extract solution volume, 1 mL

T: Reaction time, 15 minutes

C_{pr}: Sample protein concentration, mg/mL

W: Sample weight, g

N: Total number of bacteria or cells, million

10³: 1 μmol/mL = 10³ 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.