PRODUCT INFORMATION & MANUAL

Lactate Dehydrogenase Activity Assay Kit (Colorimetric)

NBP3-24511

For research use only.
Not for diagnostic or therapeutic procedures.

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Lactate Dehydrogenase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24511

Method: Colorimetric method

Specification: 100 Assays (Can detect 43 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 4 U/L

Detection range: 4-400 U/L

Average intra-assay CV (%): 2.2

Average inter-assay CV (%): 7.2

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure lactate dehydrogenase (LDH) activity in tissues, serum (plasma) and culture cells samples.

▲Background

Lactate dehydrogenase (LDH) is an oxidoreductase. LDH catalyzes the conversion of lactate to pyruvic acid and back, as it converts NAD^+ to NADH and back. LDH is composed of four subunits (tetramer). The two most common subunits are the LDH-M and LDH-H protein. LDH is released into the blood by cells after tissue damage or erythrocyte hemolysis. Extracellular LDH activity is used to detect cell damage or cell death.

▲Detection principle

Coenzyme I as a hydrogen carrier, LDH catalyzes the production of pyruvic acid from lactic acid, and pyruvic acid react with pyruvic acid to form pyruvate dinitrophenylhydrazone which is brownish red in alkaline solution. The depth of the color is proportional to the concentration of pyruvic acid. The activity of LDH can be measured indirectly by measuring the OD value at 450 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Substrate Buffer	30 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Coenzyme I	Powder × 1 vial	2-8℃ , 12 months
Reagent 3	Chromogenic Agent	30 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 4	Alkali Reagent	30 mL × 1 vial	2-8℃ , 12 months
Reagent 5	2 μmol/mL Pyruvic Acid Standard	5 mL × 1 vial	2-8℃ , 12 months
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Spectrophotometer (450 nm), Micropipettor, Vortex mixer, Water bath

Reagents

Double distilled water, Normal saline (0. 9%NaCl), PBS (0. 01M, pH 7. 4)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

1. The tissue homogenate should be determined as soon as possible to avoid repeated freezing and thawing.
2. When adding reagent 2 working solution, extend the pipette tips into the reaction solution and repeatedly suck and beat it several times, and pay attention to replace the pipette tip.
3. After adding the reagent 4 working solution, mix fully with vortex mixer, and stand at room temperature for 5 min, then measure the OD value immediately.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents besides reagent 2 to room temperature before use.
2. Preparation of reagent 2 application solution:

   Dissolve a vial of powder with 6.65 mL double distilled water and it can be stored at -20℃ for 2 weeks. It is recommended to aliquot the prepared solution and store at -20℃ .

3. Preparation of reagent 4 application solution:

   Dilute the reagent 4 with double distilled water for 10 times. Prepare fresh solution before use and it can be stored at 2-8℃ for 7 days.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (4-400 U/L).

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	10-15
Human plasma	10-15
Porcine serum	20-30
Rat serum	20-30
Rat plasma	20-30
10% Mouse kidney tissue homogenization	500-800
10% Rat lung tissue homogenization	300-500
10% Rat liver tissue homogenization	800-1000

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

▲Detailed operation steps

1. The preparation of standard curve

Dilute 2 μmol/mL pyruvic acid standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 μmol/mL. Reference is as follows:

Number	Standard concentrations (μmol/mL)	2 μmol/mL pyruvic acid standard (μL)	Double distilled water (μL)
A	0	0	1000
B	0.05	25	975
C	0.1	50	950
D	0.2	100	900
E	0.4	200	800
F	0.6	300	700
G	0.8	400	600

Assay protocol

2. The measurement of samples

(1) Standard tube: add 50 μL of double distilled water and 200 μL of pyruvic acid standard solution with different concentrations to 5 mL EP tube.
Sample tube: add 200 μL of sample to 5 mL EP tube.
Control tube: add 50 μL of double distilled water and 200 μL of sample to 5 mL EP tube.

(2) Add 250 μL of reagent 1 to each tube.

(3) Add 50 μL of reagent 2 working solution to sample tubes.

(4) Mix fully with vortex mixer and incubate at 37℃ for 15 min.

(5) Add 250 μL of reagent 3 to each tube. Mix fully with vortex mixer and incubate at 37℃ for 15 min.

(6) Add 2.5 mL of reagent 4 working solution to each tube.

(7) Mix fully with vortex mixer and stand at room temperature for 5 min. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 450 nm with 1 cm optical path cuvette.

▲Summary operation table

	Standard tube	Sample tube	Control tube
Double distilled water (μL)	50		50
Pyruvic acid standard solution with different concentrations (μL)	200		
Sample (μL)		200	200
Reagent 1 (μL)	250	250	250
Reagent 2 application solution (μL)		50	
Mix fully and incubate at 37℃ for 15 min.			
Reagent 3 (μL)	250	250	250
Mix fully and incubate at 37℃ for 15 min.			
Reagent 4 working solution (mL)	2.5	2.5	2.5
Mix fully and stand at room temperature for 5 min. Set the spectrophotometer to zero and measure the OD values of each tube.			

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. If the sample have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. The actual concentration is the calculated concentration multiplied by dilution factor.

The standard curve is: y= ax + b.

1. Serum (plasma) sample:

Unit definition: the enzyme amount of 1 μmol of pyruvic acid generated by 1 L of sample at 37℃ for 15 minutes in the reaction system is defined as 1 unit.

$$\text{LDH activity (U/L)} = (\Delta A_{450} - b) \div a \times f \times 1000^*$$

2.Tissue and cells sample:

Unit definition: the enzyme amount of 1 μmol of pyruvic acid generated by 1 g protein at 37℃ for 15 minutes in the reaction system is defined as 1 unit.

$$\text{LDH activity (U/gprot)} = (\Delta A_{450} - b) \div a \times f \div C_{pr} \times 1000^*$$

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0);

x: The concentration of standard;

a: The slope of standard curve;

b: The intercept of standard curve.

ΔA_{450}: $OD_{Sample} - OD_{Control}$.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample (gprot/L)

1000*: 1 L=1000 mL

Appendix I Data

▲Example analysis

For human serum, dilute human serum with PBS for 10 times, take 0.2 mL of diluted human serum and carry the assay according to the operation table. The results are as follows:

standard curve: y = 1.082 x + 0.0112,

the average OD value of the sample is 0.308, the average OD value of the control is 0.143, and the result is:

$$\text{LDH activity (U/gprot)} = (0.308-0.143-0.0112)\div 1.082\times 10\times 1000$$
$$= 1421.4\ \text{U/L}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum (Plasma)

Detect the sample directly. For plasma sample, heparin is recommended as an anticoagulant.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with homogenization medium at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL):the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 1500 g at 4℃ . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

▲ Cells

Collect the cells and wash the cells with homogenization medium for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4).

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/ time, interval for 3 s, the total time is 5 min).

Appendix III References

1. Holbrook J J, Liljas A, Steindel S J, et al. 4 Lactate Dehydrogenase[J]. Enzymes, 1975, 11(8): 191-292.

2. Maekawa M. Lactate dehydrogenase isoenzymes[J]. Journal of Chromatography B Biomedical Sciences & Applications, 1988, 429(429): 373-398.

3. Drent M, Cobben N A, Henderson R F, et al. Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation[J]. European Respiratory Journal, 1996, 9(8): 1736-1742.