PRODUCT INFORMATION & MANUAL

Lactate Dehydrogenase Activity Assay Kit (Colorimetric)

NBP3-24510

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

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Lactate Dehydrogenase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24510

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Instrument: Microplate reader

Sensitivity: 6 U/L

Detection range: 6-1000 U/L

Average intra-assay CV (%): 1.8

Average inter-assay CV (%): 2.4

Average recovery rate (%): 98

- ▲ 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), culture cells, hydrothorax and other samples.

▲Background

Lactate dehydrogenase (LDH or LD) is an enzyme 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. A dehydrogenase is an enzyme that transfers a hydride from one molecule to another. LDH is expressed extensively in body tissues, such as blood cells and heart muscle. Because it is released during tissue damage, it is a marker of common injuries and disease such as heart failure. Lactate dehydrogenase is composed of four subunits (tetramer). The two most common subunits are the LDH-M and LDH-H protein, encoded by the LDHA and LDHB genes, respectively. These two subunits can form five possible tetramers (isoenzymes): 4H, 4M, and the three mixed tetramers (3H1M, 2H2M, 1H3M). These five isoforms are enzymatically similar but show different tissue distribution: The major isoenzymes of skeletal muscle and liver, M4, has four muscle (M) subunits, while H4 is the main isoenzymes for heart muscle in most species, containing four heart (H) subunits.

▲Detection principle

Using coenzyme I as a hydrogen carrier, LDH catalyze lactic acid to produce pyruvate. Pyruvate reacted with 2, 4-dinitrophenylhydrazine to form pyruvate dinitrophenylhydrazone, which was red-brown in alkaline solution, and the color depth was proportional to pyruvate concentration. The activity of LDH could be calculated by measuring OD value.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Substrate Buffer	5 mL × 1 vial	2-8℃, 12 months
Reagent 2	Coenzyme I	Powder × 1 vial	2-8℃, 12 months
Reagent 3	Chromogenic Agent	5 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 4	Alkali Reagent	5 mL × 1 vial	2-8℃, 12 months
Reagent 5	2 μmol/mL Pyruvic Acid Standard	1 mL × 1 vial	2-8℃, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
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

Microplate reader(440-460nm), Micropipettor, Multichannel pipette, Incubator

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.

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 2 application solution:

   Dissolve a vial of powder with 1.33 mL double distilled water and it can be store at -20℃ for 2 weeks. It is recommended to aliquot the prepared solution and store at -20℃ .

2. Preparation of reagent 4 application solution:

   Dilute the reagent 4 with double distilled water for 10 times. Prepare fresh solution before use.

▲Sample preparation

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

Sample requirements

1. Avoid using hemolytic serum samples, as the LDH activity in red blood cells is about 100 times higher than that in serum.
2. SDS, Tween 20, NP-40, Triton X-100 and other detergents should not be included in the sample.
3. Oxalate anticoagulants should not be used because oxalate will inhibit the activity of LDH.

▲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 (6-1000 U/L).

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

Sample type	Dilution factor
Human serum	10
Human plasma	15-30
Porcine serum	10-20
Human hydrothorax	5-8
10% Mouse kidney tissue homogenization	500-800
10% Mouse lung tissue homogenization	500
10% Mouse liver tissue homogenization	500-800
HepG2 cells homogenization	100-300

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'
B	B	B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'
C	C	C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'
D	D	D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'
E	E	E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'
F	F	F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'
G	G	G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'
H	H	H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'

Note: A-H, standard wells; S1-S40, sample wells; S1'-S40', control wells

▲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, 1 μmol/mL. Reference is as follows:

Number	Standard concentrations (μmol/mL)	2 μmol/mL pyruvic acid standard solution (μL)	Double distilled water (μL)
A	0	0	400
B	0.05	10	390
C	0.1	20	380
D	0.2	40	360
E	0.4	80	320
F	0.6	120	280
G	0.8	160	240
H	1.0	200	200

2. The measurement of samples

(1) Standard well: add 5 μL of double distilled water and 20 μL of pyruvic acid standard solution with different concentrations.
Sample well: add 20 μL of Sample.
Control well: add 5 μL of double distilled water and 20 μL of sample.

(2) Add 25 μL of reagent 1 to each well.

(3) Add 5 μL of reagent 2 application solution to sample wells.

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

(5) Add 25 μL of reagent 3 to each well. Mix fully and incubate at 37℃ for 15 min.

(6) Add 250 μL of reagent 4 application solution to each well.

(7) Mix fully and stand at room temperature for 5 min. Measure the OD values of each well with microplate reader at 450 nm.

▲Summary operation table

	Standard well	Sample well	Control well
Double distilled water (μL)	5		5
Pyruvic acid standard solution with different concentrations (μL)	20		
Sample (μL)		20	20
Reagent 1 (μL)	25	25	25
Reagent 2 application solution (μL)		5	
Mix fully and incubate at 37℃ for 15 min.			
Reagent 3 (μL)	25	25	25
Mix fully and incubate at 37℃ for 15 min.			
Reagent 4 application solution (μL)	250	250	250
Mix fully and stand at room temperature for 5 min. Measure the OD value of each well.			

▲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) and other liquid 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: The absolute OD value of standard;

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

▲Notes

1. The samples should not contain decontaminant, such as SDS, Tween20, NP-40, Triton X-100 detergents.
2. As the amount of sample is small, it is recommended to operate as followings:

   (1) Use left hand to assist stably hold the pipette when sampling.

   (2) Place the pipette near the bottom of the well and slowly add samples. The tip is moved upwards when adding samples to ensure that there is minimal sample residue on the tip.

   (3) If there is a scales-type centrifuge, slow centrifugation for a few minutes is recommended to ensure that the sample and reagents gather to the bottom of the well and it helps to reduce errors.
3. Do not add reagents too fast to avoid spilling out of wells.
4. Mix the wells moderately since the wells are relatively small. The mixing should not be too violently or too gently to avoid spilling or inadequate mixing. Collecting the liquid on the well wall and shaking the well.
5. There may be differences in the initial absorbance of the plate. It is recommended to measure the initial absorbance at the corresponding wavelength before use, record the difference, and then add samples to measure.
6. The amount of coenzyme I is relatively small. Therefore it is recommended to suck the pipette tips several times in the reaction solution when adding coenzyme I. Note to replace the used pipette tips.
7. Avoid bubbles when adding samples. Break the bubbles before measurement if there are some bubbles.

Appendix I Data

▲Example analysis

Dilute human serum with PBS (0.01 M, pH 7.4) for 10 times, take 0.02 mL of diluted sample and carry the assay according to the operation table. The results are as follows:

standard curve: y = 0.75742 x +0.00789,

the average OD value of the sample is 0.232, the average OD value of the control is 0.115, and the result is:

$$\text{LDH activity (U/L)} = (0.232 - 0.115 - 0.00789) \div 0.75742 \times 10 \times 1000$$
$$= 1440.548 \text{ U/L}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲ Hydrothorax

Collect the fresh hydrothorax to the tubes with anticoagulant (heparin is recommended as an anticoagulant) and mix fully. Centrifuge the sample at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲ 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.