PRODUCT INFORMATION & MANUAL

Lactate Dehydrogenase Activity Assay Kit (Colorimetric)

NBP3-24512

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

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Lactate Dehydrogenase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24512

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.11 U/L

Detection range: 0.11-39.9 U/L

Average intra-assay CV (%): 2.3

Average inter-assay CV (%): 2.3

- 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), hydrothorax and cell 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

Lactate dehydrogenase catalyzes the reaction of lactic acid with NAD^+ to produce pyruvic acid and NADH. NADH, under the action of PMS, transfer electrons to WST-8 to produce the yellow product, which has a characteristic absorption peak at 450 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Lysis Solution	60 mL × 2 vials	-20℃ , 12 months
Reagent 2	Substrate	1.5 mL × 2 vials	-20℃ , 12 months
Reagent 3	Chromogenic Agent	1.5 mL × 2 vials	-20℃ , 12 months, shading light
Reagent 4	Coenzyme	Powder × 1 vial	-20℃ , 12 months
Reagent 5	Stop Solution	6 mL × 1 vial	-20℃ , 12 months
Reagent 6	NADH Standard	Powder × 1 vial	-20℃ , 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(450 nm), Microplate, Micropipettor, Vortex mixer, Water bath

Reagents

Double distilled water

▲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

There should be no bubbles in the wells of the microplate when measuring the OD value.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use. Preheat reagent 5 at 37°C for 20 min in advance and can be used only after it is completely clarified.
2. Preparation of reagent 4 working solution:
   Dissolve a vial of powder with 0.26 mL double distilled water. Prepare fresh solution before use and can be stored at -20°C for 1 week.
3. Preparation of reaction working solution:
   Mix reagent 2, reagent 3, reagent 4 working solution at a ratio of 12:12:1. Prepare the needed fresh solution before use and store it with shading light.
4. Preparation of 5 mmol/L standard solution:
   Dissolve a vial of powder with 2 mL double distilled water and mix fully. Prepare fresh solution before use and can be stored at -20°C for 1 week.

▲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 (0.11-39.9 U/L).

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

Sample type	Dilution factor
Human serum	10-20
Dog serum	10-20
Mouse serum	50-100
Cynomolgus monkey serum	10-20
10% Rat spleen tissue homogenate	150-250
10% Rat liver tissue homogenate	250-350
10% Rat kidney tissue homogenate	250-350
10% Rat lung tissue homogenate	250-350

Note: The diluent is reagent 1.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operating steps

1. The preparation of standard curve

Dilute 5 mmol/L NADH standard with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 400, 300, 250, 200, 150, 100, 50, 0 μmol/L. Reference is as follows:

Number	Standard concentrations (μmol/L)	5 mmol/L Standard (μL)	Reagent 1 (μL)
A	0	0	1000
B	50	10	990
C	100	20	980
D	150	30	970
E	200	40	960
F	250	50	950
G	300	60	940
H	400	80	920

2. The measurement of samples

(1) Standard well: Take 50 μL of standard solution with different concentrations into the corresponding wells.
Sample well: Take 50 μL of sample into the corresponding wells.

(2) Add 50 μL of reaction working solution to each well.

(3) Incubate at 37℃ for 10 min.

(4) Add 50 μL of reagent 5 to each well.

(5) Mix fully for 5 s with microplate reader. Measure the OD values of each well with microplate reader at 450 nm.

▲Summary operation table

	Standard well	Sample well
Sample (μL)		50
Standard solution with different concentrations (μL)	50	
Reaction working solution (μL)	50	50
Incubate at 37℃ for 10 min		
Reagent 5 (μL)	50	50
Mix fully and measure the OD values at 450 nm.		

▲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 NADH generated by 1 L of liquid sample per minute at 37℃ is defined as 1 unit.

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

2.Tissue and cells sample:

Unit definition: the enzyme amount of 1 μmol of NADH generated by 1 g tissue protein or cell protein per minute at 37℃ is defined as 1 unit.

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

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}: $OD_{Sample} - OD_{Blank}$.

f: Dilution factor of sample before test.

T: Reaction time (10 min)

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

▲Example analysis

Take 50 μL of human serum diluted for 10 times and carry the assay according to the operation table. The results are as follows:

standard curve: y = 0.0027 x – 0.0178,

the average OD value of the sample is 0.229, the average OD value of the blank is 0.054, and the result is:

$$\text{LDH activity (U/L)} = (0.229-0.054+0.0178) \div 0.0027 \div 10 \times 10$$
$$= 71.4 \text{ U/L}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum (Plasma)

Detect the sample directly.

▲ 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.

▲ 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 (NBP3-25873).

▲ 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 (NBP3~25873).

Note:

1. Homogenized medium: Reagent 1.
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 nstrument (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).