PRODUCT INFORMATION & MANUAL

L-Lactic Acid Assay Kit (Colorimetric)

NBP3-25875

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

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L-Lactic Acid Assay Kit (Colorimetric)

Catalog No: NBP3-25875

Method: Colorimetric method

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

Measuring instrument: Microplate reader

Sensitivity: 0.10 mmol/L

Detection range: 0.12-7.0 mmol/L

Average intra-assay CV (%): 1.4

Average inter-assay CV (%): 3.5

Average recovery rate (%): 105

- ▲ 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 lactic acid (LA) content in serum (plasma), tissue, cells, culture supernatant samples.

▲Background

Lactic acid is an intermediate product of glucose metabolism in the body, which is mainly produced by red blood cells, striated muscle and brain tissue. The concentration of lactic acid in the blood mainly depends on the synthesis speed and metabolic rate of liver and kidney. The bidirectional conversion of lactic acid and pyruvate is regulated by lactate dehydrogenase (LDH).

▲Detection principle

Using NAD^+ as H^+ receptor, LDH catalyzes the reaction of lactic acid and NAD^+ to generate pyruvic acid and NADH respectively. NBT is reduced to a kind of purple compound during the reaction. Measure the OD value at 530 nm, and the concentration of lactic acid can be calculated.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	6 mL × 2 vials	2-8℃ , 12 months
Reagent 2	Enzyme Stock Solution	0.06 mL × 2 vials	2-8℃ , 12 months
Reagent 3	Chromogenic Agent	1.2 mL × 2 vials	2-8℃ , 12 months, shading light
Reagent 4	Stop Solution	12 mL × 2 vials	2-8℃ , 12 months
Reagent 5	10 mmol/L Lactic Acid Standard	1 mL × 2 vials	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 (520-540 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, 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. Severe hemolysis or jaundice may raise the OD value.
2. Prevent the formulation of bubbles when adding the liquid to the microplate.

Pre-assay preparation

▲Reagent preparation

Preparation of chromogenic reagent

Mix reagent 1, reagent 2, reagent 3 at the volume ratio of 100: 1: 20 fully. Prepare the fresh solution before use.

▲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 (0.12-7.0 mmol/L).

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

Sample type	Dilution factor
Human serum	2-5
10% Rat kidney tissue homogenate	1-3
10% Rat brain tissue homogenate	1
HepG2 cell culture supernatant	1
HepG2 cells	1

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

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 operation steps

1. The preparation of standard curve

Dilute 10 mmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 3, 4, 5, 6, 7 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	10 mmol/L standard (μL)	Double distilled water (μL)
A	0	0	200
B	1	20	180
C	2	40	160
D	3	60	140
E	4	80	120
F	5	100	100
G	6	120	80
H	7	140	60

2. The measurement of samples

1) Standard well: add 5 μL of standards with different concentrations to the corresponding wells.

   Sample well: add 5 μL of sample to the corresponding wells.

2) Add 120 μL of chromogenic reagent to each well.

3) Mix fully and incubate at 37℃ for 5 min.

4) Add 180 μL of reagent 4 to each well.

5) Mix fully for 5 s with microplate reader. Measure the OD values of each well at 530 nm with microplate reader.

▲Summary operation table

	Standard well	Sample well
Standards with different concentrations (μL)	5	
Sample (μL)		5
Chromogenic reagent (μL)	120	120
Mix fully and incubate at 37℃ for 5 min.		
Reagent 4 (μL)	180	180
Mix fully. Measure the OD values 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. The standard curve is: y= ax + b.

1. For serum (plasma), culture supernatant and other liquid sample:

$$\text{LA content (mmol/L)} = (\Delta A_{530} - b) \div a \times f$$

2. For tissue and cells sample:

$$\text{LA content (mmol/gprot)} = (\Delta A_{530} - b) \div a \div C_{pr} \times f$$

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_{530}: Absolute OD ($OD_{Sample} - OD_{Blank}$).

f: Dilution factor of sample before test.

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

Appendix I Data

▲Example analysis

Dilute the human serum with double distilled water for 5 times, take 5 μL of diluted sample and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 0.266 x – 0.065, the average OD value of the sample is 0.414, the average OD value of the blank well is 0.052, and the calculation result is:

$$\text{LA content (mmol/L)} = \frac{0.414 - 0.052 + 0.065}{0.266} \times 5 = 8.03 \text{ mmol/L}$$

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, 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.

▲ Cell culture supernatant

Collect fresh cell culture supernatant and centrifuge at 10000 g for 10 min at 4℃ . Take the supernatant to preserve it on ice for detection.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) 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 10000 g at 4 °C . 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 PBS (0.01 M, pH 7.4) 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: Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm3), 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. Gladden L B. Lactate metabolism: a new paradigm for the third millennium. Journal of Physiology, 2004, 558(1): 5-30.
2. Doherty J R, Cleveland J L. Targeting lactate metabolism for cancer therapeutics. Journal of Clinical Investigation, 2013, 123(9): 3685-3692.