PRODUCT INFORMATION & MANUAL

L-Lactic Acid Assay Kit (Colorimetric)

NBP3-25876

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

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L-Lactic Acid Assay Kit (Colorimetric)

Catalog No: NBP3-25876

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.05 mmol/L

Detection range: 0.05-6.0 mmol/L

Average intra-assay CV (%): 1.1

Average inter-assay CV (%): 1.9

Average recovery rate (%): 101

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

▲Background

Lactic acid is the intermediate product of glucose metabolism in vivo, which is mainly produced by erythrocytes, striated muscle and brain tissues. The concentration of lactic acid in blood depends on the synthesis and metabolic rate of liver and kidney.

▲Detection principle

Using NAD^+ as hydrogen acceptor, LDH catalyzes the conversion of both lactate and NAD^+ into pyruvic acid and NADH respectively. 1-Methoxy-5-methyl phenazine methyl sulfate (PMS) transfers hydrogen from NADH to NBT which deoxidize into purple chromogenic substrate. Lactic acid content can be calculated by measuring the OD value at 530 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 2 vials	2~8℃ , 12 months
Reagent 2	Enzyme Stock Solution	1.2 mL × 1 vial	2~8℃ , 12 months
Reagent 3	Chromogenic Agent	24 mL × 1 vial	2~8℃ , 12 months, shading light
Reagent 4	Stop Solution	60 mL × 4 vials	2~8℃ , 12 months
Reagent 5	3 mmol/L Lactic Acid Standard	2 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 (530 nm), Micropipettor, Vortex mixer, Incubator, Centrifuge

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. The time of reaction time must be accurate.
2. The assay must be completed within 30 minutes after adding reagent 4.

Pre-assay preparation

▲ Reagent preparation

Preparation of the enzyme working solution

Mix reagent 2 and reagent 1 at the ratio of 1:100 before use. Prepare the fresh solution before use and it can be stored at 4℃ for 24 hours.

▲ 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 (0.05-6.0 mmol/L).

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

Sample type	Dilution factor
Human serum	4-8
10% Mouse muscle tissue homogenization	2-4
10% Mouse liver tissue homogenization	1
Rat serum	4-8
HePG2 cells homogenization (1.388 gprot/L)	4-8
HepG2 supernatant	2-4

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

Assay protocol

▲Detailed operation steps

1. Blank tube: add 20 μL of double distilled water to the 5 mL EP tube.
   Standard tube: add 20 μL of 3 mmol/L Lactic Acid Standard to the 5 mL EP tube.
   Sample tube: add 20 μL mL of sample to the 5 mL EP tube.
2. Add 1000 μL of enzyme working solution and 200 μL of reagent 3 and oscillate fully.
3. Incubate the tubes at 37℃ for 10 min accurately.
4. Add 2000 μL of reagent 4 and mix fully.
5. Set the spectrometer to zero with double distilled water and measure the OD value of each tube at 530 nm with 1 cm optical path cuvette. (Avoid bubbles when measuring the OD values and read the results within 30 min.)

▲Summary operation table

	Blank tube	Standard tube	Sample tube
ddH_2O (μL)	20		
3 mmol/L Standard (μL)		20	
Sample (μL)			20
Enzyme working solution (μL)	1000	1000	1000
Reagent 3 (μL)	200	200	200
Mix fully and incubate in 37℃ for 10 min accurately.			
Reagent 4 (μL)	2000	2000	2000
Mix fully, set spectrophotometer to zero and measure the OD values of each tube.			

▲Calculation

Serum (plasma) and other liquid sample:

$$\text{Lactic acid content (mmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

Tissue and cells sample:

$$\text{Lactic acid content (mmol/gprot)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div C_{pr}$$

Note:

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: Concentration of standard, 3 mmol/L

f: Dilution factor of sample before test

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

Appendix I Data

▲Example analysis

Dilute human serum with double distilled water for 5 times, take 0.02 mL of diluted sample and carry the assay according to the operation table.

The results are as follows:

The average OD value of the sample is 0.363, the average OD value of the blank is 0.075, the average OD value of the standard is 0.491, the concentration of standard is 3 mmol/L, and the calculation result is:

$$\text{Lactic acid content (mmol/L)} = \frac{0.363-0.075}{0.491-0.075} \times 3 \times 5 = 10.38 \text{ mmol/L}$$

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.

▲Tissue sample

Take 0.02-1g fresh tissue to wash with homogenization medium at 2-8℃ to remove blood cells. 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℃ . 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 minat 4℃ , 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.

▲Cell culture supernatant

Centrifuge at 10000 g for 10 min at 4℃ and take the supernatant to preserve it on ice for detection. If not detected on the same day, it can be stored at -80℃ for a month.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4) or 0.9% NaCl.

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. 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.