PRODUCT INFORMATION & MANUAL

L-Lactic Acid Assay Kit (Colorimetric)

NBP3-25877

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

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Novus kits are guaranteed for 6 months from date of receipt

L-Lactic Acid Assay Kit (Colorimetric)

Catalog No: NBP3-25877

Method: Colorimetric method

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

Instrument: Spectrophotometer

Sensitivity: 0.14 mmol/L

Detection range: 0.14-7.0 mmol/L

Average intra-assay CV (%): 1.6

Average inter-assay CV (%): 2

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 the L-lactic acid (LA) content in whole blood samples.

▲Background

Lactic acid is the intermediate product of glucose metabolism in vivo, which is mainly produced by erythrocytes, rhabdomytes and brain tissues. The concentration of lactate in blood depends on the synthesis speed and metabolic rate of liver and kidney. The bidirectional transformation of lactic acid and pyruvate is regulated by lactate dehydrogenase (LDH).

▲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	Protein Precipitator	Powder × 2 vials	2-8℃ , 12 months
Reagent 2	Enzyme Diluent	60 mL × 2 vials	2-8℃ , 12 months
Reagent 3	Enzyme Stock Solution	1.2 mL × 1 vial	2-8℃ , 12 months
Reagent 4	Chromogenic Agent	24 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 5	3 mmol/L Lactic Acid Standard	1 mL ×1 vial	2-8℃ , 12 months
Reagent 6	Stop Solution	60 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.
3. When the reagent 3 is used up, please timely store it at 2-8℃ .

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 1 working solution

Dissolve a vial of reagent 1 with 35 mL of double distilled water fully (If there is no dissolved floating substance, do not affect the usage). The prepared solution can be stored at 2-8℃ for 6 months

2. Preparation of enzyme working solution

Mix reagent 2 and reagent 3 at a ratio of 100: 1 fully. Prepare the fresh solution before use and operate on ice. The prepared solution can be store at 2-8℃ for 24 hours.

3. Preparation of reagent 6 working solution

Dilute the reagent 6 with double distilled water at a ratio of 1: 3. Prepare the fresh solution before use.

▲Sample preparation

1. Collect the whole blood sample

Take fresh blood to the tube containing the anticoagulant and gently mix it upside downand. Preserve it on ice for detection.

2. The pretreatment of sample

Add reagent 1 working solution to whole blood sample according to the ratio of whole blood (mL): reagent 1 working solution (mL)=1: 6 (for example, add 0.3 mL of reagent 1 working solution to 0.05 mL of whole blood sample). Then centrifuge at 1100 g for 10 min. Take the supernatant for detection.

▲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 (0.14-7.0 mmol/L).

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

Sample type	Dilution factor
Rabbit whole blood	1-2
Mouse whole blood	1-2
Rat whole blood	1-2

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

Assay protocol

▲Operating 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 a 5 mL EP tube.
2. Add 1000 μL of enzyme working solution and 200 μL of reagent 4 and oscillate fully.
3. Incubate the tubes at 37℃ for 10 min.
4. Add 2000 μL of reagent 6 working solution 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.)

▲Operation table

	Blank tube	Standard tube	Sample tube
Double distilled water (μL)	20		
3 mmol/L Lactic Acid Standard (μL)		20	
Sample supernatant (μL)			20
Enzyme working solution (μL)	1000	1000	1000
Reagent 4 (μL)	200	200	200
Mix fully and incubate at 37℃ water bath for 10 min.			
Reagent 6 working solution (μL)	2000	2000	2000
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.			

▲Calculation

$$\text{LA content (mmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times 7^{*} \times f$$

Note:

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: Concentration of standard, 3 mmol/L

7^{*}: Dilution factor in the pretreatment of sample

f: Dilution factor of sample before test

Appendix I Data

▲Example analysis

Take 0.1 mL of rabbit whole blood sample, add 0.6 mL of reagent 1, mix fully and centrifuge at 1100 g for 10 min. Take 0.02 mL of supernatant and carry the assay according to the operation table.

The results are as follows:

The average OD value of the sample is 0.247, the average OD value of the blank is 0.103, the average OD value of the standard is 0.520, the concentration of standard is 3 mmol/L, and the calculation result is:

$$\text{Lactic Acid content (mmol/L)} = \frac{0.247-0.103}{0.520-0.103} \times 3\ \text{mmol/L} \times 7 = 7.25\ \text{mmol/L}$$

Appendix II 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.