（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K043-S

Specification：50 Assays(48 samples)/ 100 Assays(96 samples)

Measuring instrument：Spectrophotometer (530 nm)

Detection range：0.14-7.0 mmol/L

Elabscience® L-Lactic Acid (LA) Colorimetric Assay Kit (Whole Blood Samples)

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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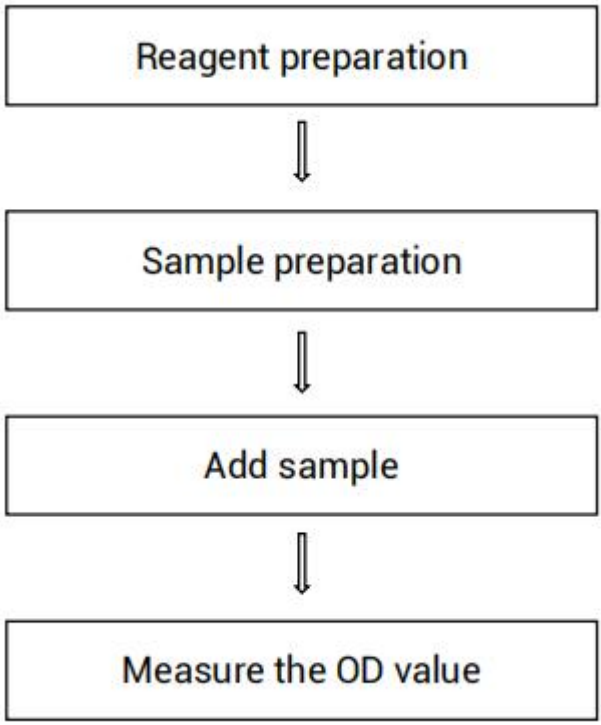
Assay summary



Intended use

This kit can be used to measure the L-lactic acid (LA) content in whole blood samples.

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	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Protein Precipitator	Powder × 1 vial	Powder × 2 vials	2-8℃, 12 months
Reagent 2	Enzyme Diluent	60 mL × 1 vial	60 mL × 2 vials	2-8℃, 12 months
Reagent 3	Enzyme Stock Solution	0.6 mL × 1 vial	1.2 mL × 1 vial	2-8℃, 12 months
Reagent 4	Chromogenic Agent	12 mL × 1 vial	24 mL × 1 vial	2-8℃, 12 months shading light
Reagent 5	3 mmol/L Standard Solution	1 mL ×1 vial	1 mL ×1 vial	2-8℃, 12 months
Reagent 6	Stop Solution	30 mL × 1 vial	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Spectrophotometer (530 nm), Micropipettor, Vortex mixer, Incubator, Centrifuge

Reagents:

Double distilled water, Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)

Reagent preparation

① Keep enzyme stock solution on ice during use. Equilibrate other reagents to room temperature before use.

② The preparation of protein precipitator working solution：
Dissolve one vial of protein precipitator with 35 mL of double distilled water, mix well to dissolve (If there is no dissolved floating substance, do not affect the usage). Store at 2-8℃ for 6 months.

③ The preparation of enzyme working solution：
For each tube, prepare 1000 μL of enzyme working solution (mix well 990 μL of enzyme diluent and 10 μL of enzyme stock solution). The enzyme working solution should be prepared on spot and operate on ice. Store at 2-8℃ for 24 hours.

④ The preparation of stop working solution：
For each tube, prepare 2000 μL of stop working solution (mix well 500 μL of stop solution and 1500 μL of double distilled water). The stop working solution should be prepared on spot.

Sample preparation

① Sample preparation

Whole blood sample:

① Take fresh blood to the tube containing the anticoagulant and gently mix it upside downand. Preserve it on ice for detection. If not detected on the same day, the serum can be stored at 4℃ for 2 days.

② Add 0.3 mL of protein precipitator working solution to 0.05 mL of whole blood sample.

③ Centrifuge at 1100×g for 10 min at 4℃ to remove insoluble material. Collect supernatant for detection.

② Dilution of sample

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). For the dilution of other sample types, please do pretest to confirm the dilution factor

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 chromogenic agent.

③ When the enzyme stock solution is used up, please timely store it at 2-8℃.

Operating steps

① Blank tube: add 20 μL of double distilled water to the 5 mL EP tube.
Standard tubes: add 20 μL of 3 mmol/L lactic acid Standard to the 5 mL EP tube.
Sample tubes: add 20 μL mL of sample to the 5 mL EP tube.

② Add 1000 μL of enzyme working solution and 200 μL of chromogenic agent and oscillate fully.

③ Incubate the tubes at 37℃ for 10 min.

④ Add 2000 μL of stop working solution and mix well.

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.).

Calculation

The sample:

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

[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 Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rabbit whole blood samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	1.30	3.40	5.70
%CV	1.8	1.5	1.5

Inter-assay Precision

Three rabbit whole blood samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	1.30	3.40	5.70
%CV	2.1	2.3	1.6

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 101%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	2.6	4.8	6.1
Observed Conc. (mmol/L)	2.6	4.8	6.3
Recovery rate (%)	99	100	104

Sensitivity

The analytical sensitivity of the assay is 0.14 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix Π Example Analysis

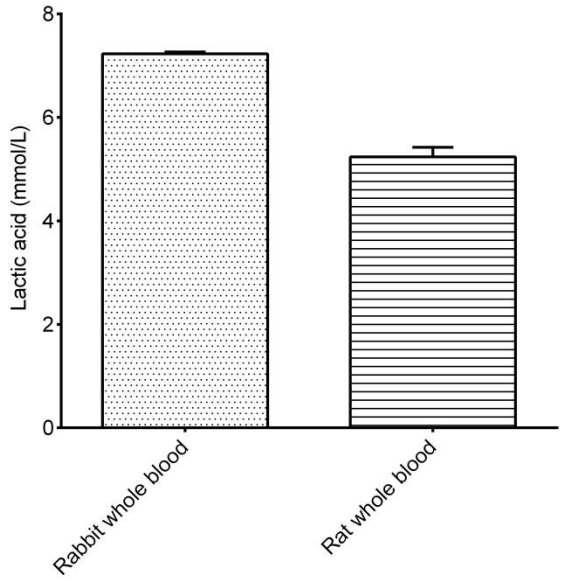
Example analysis：

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 steps. 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:

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

Detect rabbit whole blood (dilute for 2 times, V=50 μL), rat whole blood (V=50 μL), according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.