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

Catalog No：E-BC-K044-S

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

Measuring instrument：Spectrophotometer（530 nm）

Detection range：0.05-6.0 mmol/L

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

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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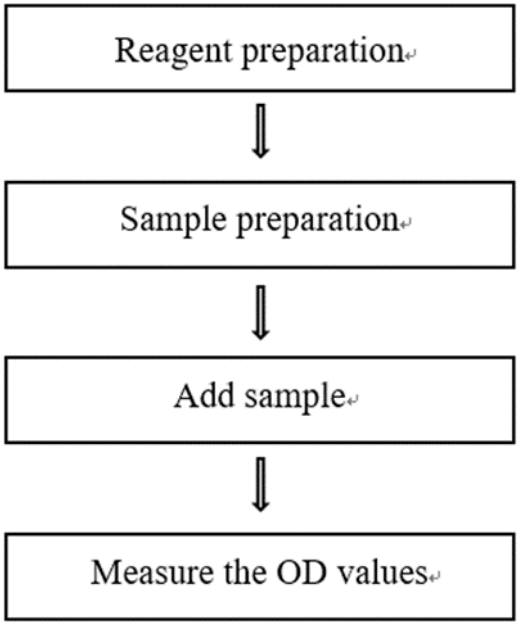
Assay summary



Intended use

This kit can be used to measure L-lactic acid (LA) content in tissue, serum (plasma), cells and culture supernatant 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	Buffer Solution	60 mL ×1 vial	60 mL ×2 vials	2-8℃, 12 months
Reagent 2	Enzyme Stock Solution	0.6 mL ×1 vial	1.2 mL ×1 vial	2-8℃, 12 months
Reagent 3	Chromogenic Agent	12 mL ×1 vial	24 mL ×1 vial	2-8℃, 12 months shading light
Reagent 4	Stop Solution	60 mL ×2 vials	60 mL ×4 vials	2-8℃, 12 months
Reagent 5	3 mmol/L Lactic Acid Standard	2 mL ×1 vial	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. 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 enzyme working solution:

For each tube, prepare 1000 μL of enzyme working solution (mix well 990 μL of buffer solution 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.

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or 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:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4 ℃.

④ Centrifuge at 10000 ×g for 10 min at 4 ℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cells:

① Harvest the number of cells needed for each assay (initial recommendation 10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 10^6 cells in 300-500 μL PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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

Sample type	Dilution factor
Human serum	4-8
Rat serum	4-8
10% Mouse muscle tissue homogenization	2-4
10% Mouse liver tissue homogenization	1
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). 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 stop solution.

Operating steps

① Blank tube: add 20 μL of double distilled water into a 5 mL EP tube.
Standard tubes: add 20 μL of 3 mmol/L Lactic Acid Standard into a 5 mL EP tube.
Sample tubes: add 20 μL mL of sample into a 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 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:

1. Serum (plasma) and other liquid sample:

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

2. 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 Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum 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)	0.65	2.40	5.60
%CV	1.3	1.0	1.0

Inter-assay Precision

Three human serum 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)	0.65	2.40	5.60
%CV	1.8	2.0	1.9

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)	1.5	3.6	4.8
Observed Conc. (mmol/L)	1.5	3.6	4.9
Recovery rate (%)	99	101	103

Sensitivity

The analytical sensitivity of the assay is 0.05 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 II Example Analysis

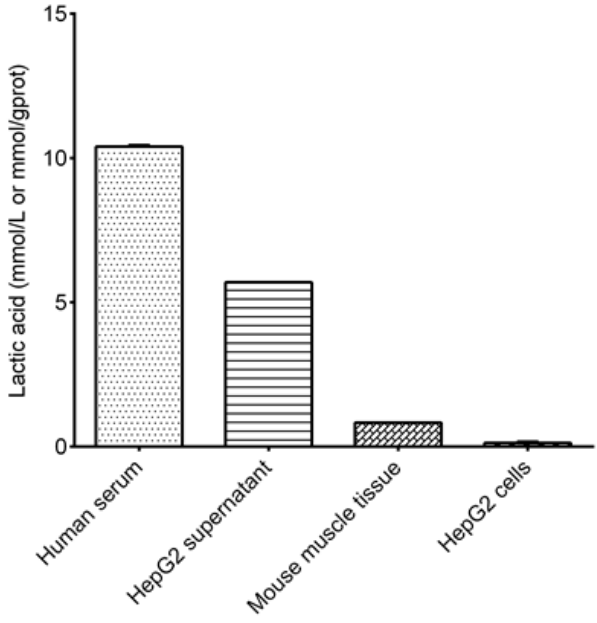
Example analysis:

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 steps. 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:

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

Detect human serum (dilute for 5 times), 10% mouse muscle tissue homogenate (the concentration of protein in sample is 4.540 gprot/L, dilute for 2 times), HepG2 cells supernatant (dilute for 3 times) and HepG2 cells (the concentration of protein in sample is 1.388 gprot/L), 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.