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

Catalog No：E-BC-K044-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader (520-540 nm)

Detection range：0.12-7.0 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
Tel: 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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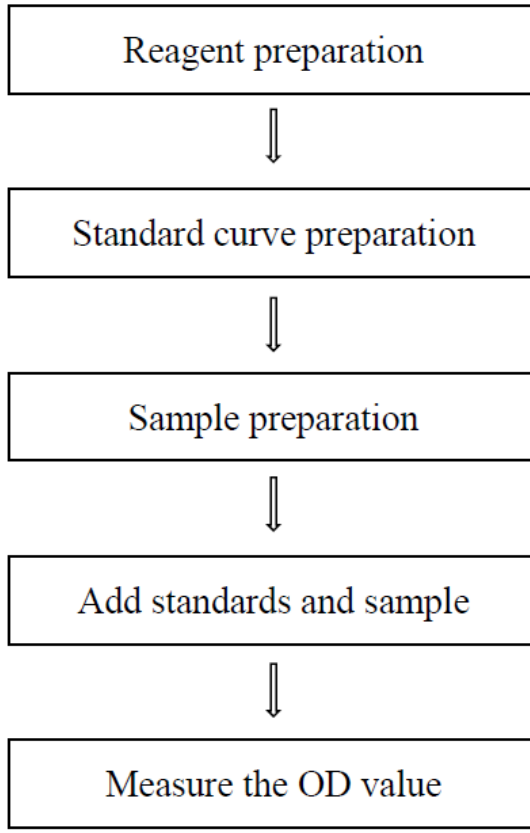
Assay summary



Intended use

This kit can be used to measure lactic acid (LA) content in serum (plasma), tissue, cells, culture supernatant samples.

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	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	6 mL ×1 vial	6 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	1.2 mL ×1 vial	1.2 mL ×2 vials	2-8℃, 12 months shading light
Reagent 4	Stop Solution	12 mL ×1 vial	12 mL ×2 vials	2-8℃, 12 months
Reagent 5	10 mmol/L Lactic Acid Standard	1 mL ×1 vial	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (520-540 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

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:

Before testing, please prepare sufficient enzyme working solution according to the test wells. For example, prepare 110 μL of enzyme working solution (mix well mix well 100 μL of buffer solution and 10 μL of enzyme stock solution). The enzyme working solution should be prepared on spot.

③ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 10 mmol/L standard with deionized 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:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
10 mmol/L standard (μL)	0	20	40	60	80	100	120	140
Deionized water (μL)	200	180	160	140	120	100	80	60

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 3×10^6-5×10^6 cells).

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

③ Homogenize 3×10^6-5×10^6 cells in 200 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	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) 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 Severe hemolysis or jaundice may raise the OD value.

② Prevent the formulation of bubbles when adding the liquid to the microplate.

Operating steps

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 100 μL of enzyme working solution to each well.

③ Add 20 μL of chromogenic agent to each well.

④ Incubate at 37℃ for 10 min.

⑤ Add 180 μL of stop solution to each well.

⑥ Mix well for 5 s with microplate reader. Measure the OD values of each well at 530 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

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

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

2. Tissue and cells sample:

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

[Note]

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

f: Dilution factor of sample before test.

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

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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)	0.50	2.10	4.60
%CV	1.8	1.2	1.2

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)	0.50	2.10	4.60
%CV	3.1	3.4	4.0

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 105%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	1.5	3.6	5.4
Observed Conc. (mmol/L)	1.6	3.7	5.7
Recovery rate (%)	106	103	106

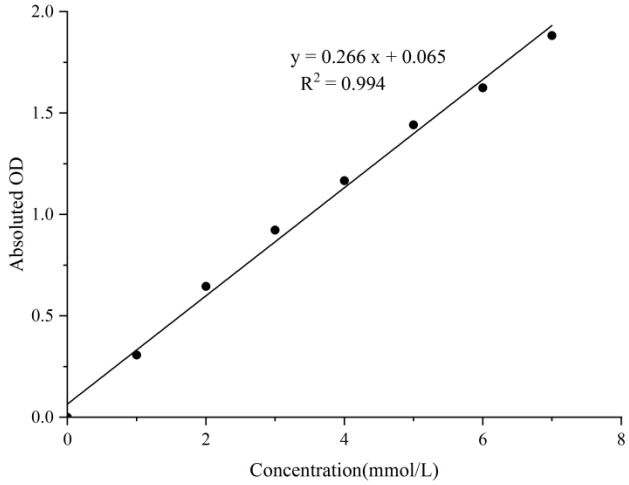
Sensitivity

The analytical sensitivity of the assay is 0.10 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.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mmol/L)	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Average OD	0.160	0.466	0.805	1.082	1.327	1.601	1.784	2.042
Absoluted OD	0.000	0.307	0.646	0.923	1.167	1.442	1.624	1.882



Appendix II Example Analysis

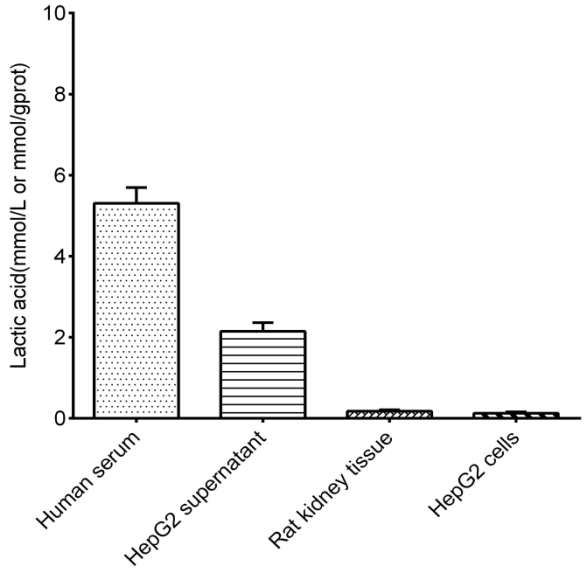
Example analysis:

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 steps. 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:

$$\text{LA content (mmol/L)} = (0.414 - 0.052 - 0.065) \div 0.266 \times 5 = 5.58 \text{ mmol/L}$$

Detect human serum (dilute for 5 times), culture supernatant of HepG2 cells, 10% rat kidney tissue homogenate (the concentration of protein is 7.18 gprot/L), and HepG2 cells (the concentration of protein is 9.55 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.