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

Catalog No：E-BC-K002-M

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

Measuring instrument：Microplate reader（520-550 nm）

Detection range：0.06-8.0 mmol/L

Elabscience®D-Lactic Acid/Lactate (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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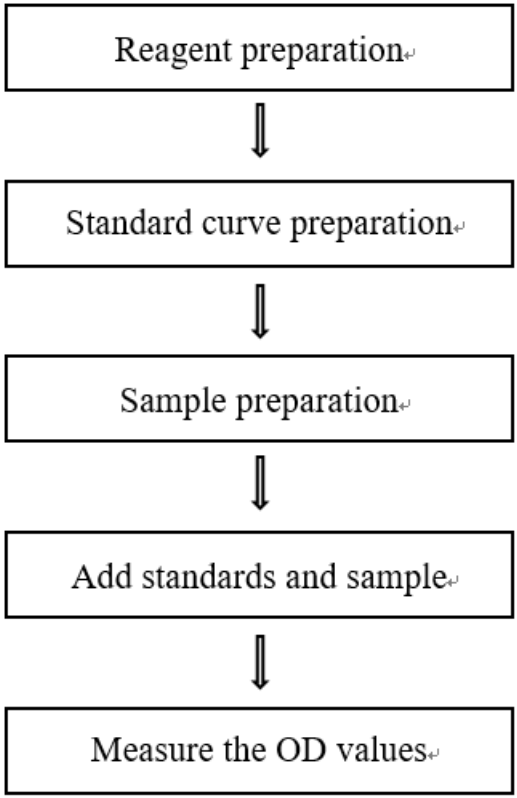
Assay summary



Intended use

This kit can be used to measure D-lactic acid (LA) content in tissue, serum (plasma) and saliva samples.

Detection principle

Using NAD^+ as H^+ receptor, D-lactate dehydrogenase (LDH) catalyzes the reaction of D-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 D-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	12 mL ×1 vial	2-8℃, 12 months
Reagent 2	Enzyme Stock Solution	0.06 mL ×1 vial	0.12 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	24 mL ×1 vial	2-8℃, 12 months
Reagent 5	10 mmol/L Standard Solution	1.0 mL ×1 vial	2.0 mL ×1 vial	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:

Micropipettor, Vortex mixer, Incubator, Centrifuge, Microplate reader (520-550 nm, optimum wavelength: 530 nm)

Reagent preparation

① Keep enzyme stock solution on ice for use. Equilibrate all the reagents to room temperature before use.

② Preparation of enzyme working solution：

Before testing, please prepare sufficient enzyme working solution according to the test wells. For example, prepare 505 μL of enzyme working solution (mix well 5 μL of enzyme stock solution and 500 μL of buffer 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 solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 4, 5, 6, 7, 8 mmol/L. Reference is as follows:

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

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.

Saliva: Gargle with clear water, collect the saliva 30 min later, centrifuge at 10000 g for 10 min at 4℃. Take the supernatant and 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 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 plasma	1
Human serum	1
Rat serum	1
Rat plasma	1
Mouse serum	1
Rabbit serum	1
10% Rat kidney tissue homogenate	2-3
10% Mouse brain tissue homogenate	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.
③ If the D-lactic acid content is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (E-BC-K318-M).

Operating steps

① Standard well: add 5 μL of standards with different concentrations into the standard wells.

Sample well: add 5 μL of sample into the sample wells.

② Add 100 μL of enzyme working solution to each well.

③ Add 20 μL of chromogenic agent to each well.

④ Mix fully and incubate at 37℃ for 10 min.

⑤ Add 180 μL of stop solution to each well.

⑥ Mix fully 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) sample:

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

2. Tissue sample:

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

[Note]

ΔA_{530}: $OD_{Sample} - OD_{Blank}$

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)	0.50	2.50	5.00
%CV	4.2	3.6	3.6

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	2.50	5.00
%CV	8.2	7.5	7.4

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 99%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	1.5	4.5	6.5
Observed Conc. (mmol/L)	1.5	4.4	6.4
Recovery rate (%)	101	98	98

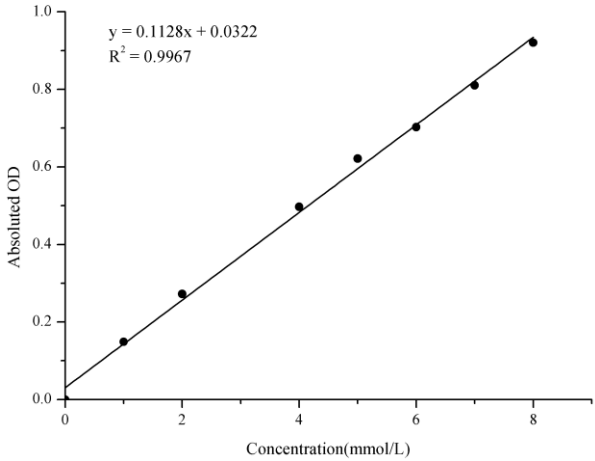
Sensitivity

The analytical sensitivity of the assay is 0.06 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**	**1.0**	**2.0**	**4.0**	**5.0**	**6.0**	**7.0**	**8.0**
OD value	0.138	0.284	0.414	0.648	0.752	0.856	0.934	1.083
	0.136	0.288	0.405	0.620	0.765	0.823	0.961	1.032
Average OD	0.137	0.286	0.410	0.634	0.759	0.840	0.948	1.058
Absoluted OD	0.000	0.149	0.273	0.497	0.622	0.703	0.811	0.921



Appendix II Example Analysis

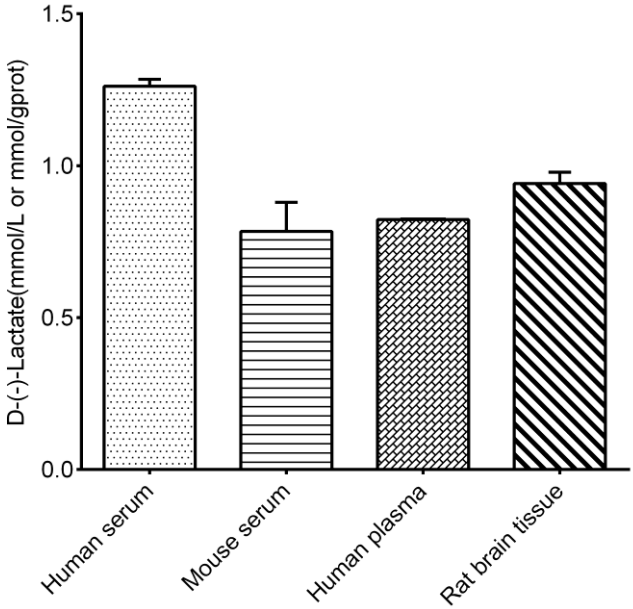
Example analysis:

For human serum, take 5 μL of human serum and carry the assay according to the operation table. The results are as follows:

standard curve: y = 0.1082 x + 0.0232, the average OD value of the sample is 0.307, the average OD value of the blank is 0.148, and the calculation result is:

D-LA content (mmol/L) = (0.307- 0.148 – 0.0232) ÷0.1082 = 1.26 mmol/L

Detect human serum, mouse serum, human plasma and 10% rat brain tissue homogenate (the concentration of protein is 3.64 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.