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

Catalog No：E-BC-K046-S

Specification：100 Assays (43 samples)

Measuring instrument：Microplate reader（440-460 nm）

Detection range：4-400 U/L

Elabscience®Lactate Dehydrogenase (LDH) Activity Assay Kit

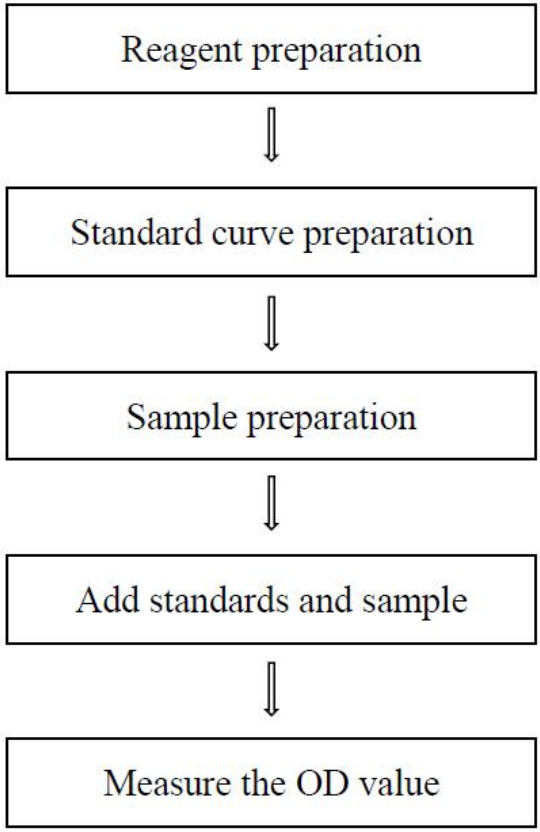
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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.

Table of contents

Assay summary



Intended use

This kit can be used to measure Lactate dehydrogenase (LDH) activity in serum (plasma), tissues and cells samples.

Detection principle

Coenzyme I as a hydrogen carrier, LDH catalyzes the production of pyruvic acid from lactic acid, pyruvic acid react with 2, 4-dinitrophenylhydrazine to form pyruvate dinitrophenylhydrazone which is brownish red in alkaline solution. The depth of the color is proportional to the concentration of pyruvic acid. The activity of LDH can be measured indirectly by measuring the OD value at 450 nm.

Kit components & storage

Item	Component	Size (100 Assays)	Storage
Reagent 1	Substrate Buffer	30 mL × 1 vial	2-8°C, 12 months
Reagent 2	Coenzyme I	Power × 1 vial	2-8°C, 12 months
Reagent 3	Chromogenic Agent	30 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 4	Alkali Reagent	30 mL × 1 vial	2-8°C, 12 months
Reagent 5	2 μmol/mL Pyruvic Acid Standard	5 mL × 1 vial	2-8°C, 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:

Micropipettor, Vortex mixer, Water bath, Spectrophotometer (450 nm)

Reagents:

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

Reagent preparation

① Keep coenzyme I on ice during use. Equilibrate other reagents to room temperature before use.

② The preparation of coenzyme I application solution:

Dissolve one vial of coenzyme I with 6.65 mL of double distilled water. Mix well to dissolve. Store at 2-8 °C for 15 days or aliquoted storage at -20°C for 1 month.

③ The preparation of alkali application solution:

For each well, prepare 2500 μL of alkali application solution (mix well 250 μL of alkali reagent and 2250 μL of double distilled water). The alkali application solution should be prepared on spot. Store at 2-8 °C for 7 days.

④ The preparation of standard curve:

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

Dilute 2 μmol/mL pyruvic acid standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 μmol/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦
Concentration (μmol/mL)	**0**	**0.05**	**0.1**	**0.2**	**0.4**	**0.6**	**0.8**
2 μmol/mL standard (μL)	0	25	50	100	200	300	400
Double distilled water (μL)	1000	975	950	900	800	700	600

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°C for a month.

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 Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4° C.

④ Centrifuge at 10000×g for 10 minutes 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).

Cell (adherent or suspension) sample:

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

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

③ Homogenize 1×10^6 cells in 300 μL-500 μL normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10000×g for 10 minutes 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	10-15
Human plasma	10-15
Porcine serum	20-30
Rat serum	20-30
Rat plasma	20-30
10% Rat liver tissue homogenate	800-1000
10% Mouse kidney tissue homogenate	500-800
10% Rat lung tissue homogenate	300-500

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 tissue homogenate should be determined as soon as possible to avoid repeated freezing and thawing.

② It is recommended to suck the pipette tips several times in the reaction solution when adding coenzyme I application solution. Note to replace the used pipette tips.

③ After adding alkali application solution, mix fully with vortex mixer, and stand at room temperature for 5 min, then measure the OD value immediately.

Operating steps

① Standard tube: add 50 μL of double distilled water and 200 μL of pyruvic acid standard solution with different concentrations to 5 mL EP tube.
Sample tube: add 200 μL of sample to 5 mL EP tube.
Control tube: add 50 μL of double distilled water and 200 μL of sample to 5 mL EP tube.

② Add 250 μL of substrate buffer to each tube.

③ Add 50 μL of coenzyme I application solution to sample tube.

④ Mix fully and incubate at 37°C for 15 min.

⑤ Add 250 μL of chromogenic agent to each tube. Mix fully and incubate at 37°C for 15 min.

⑥ Add 2500 μL of alkali application solution to each tube.

⑦ Mix fully with vortex mixer and stand at room temperature for 5 min. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 450 nm with 1 cm optical path cuvette.

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:

Unit definition: the enzyme amount of 1 μmol of pyruvic acid generated by 1 L of sample at 37°C for 15 minutes in the reaction system is defined as 1 unit.

$$\text{LDH activity (U/L)} = (\Delta A_{450} - b) \div a \times f \times 1000^*$$

2. Tissue and cells sample:

Unit definition: the enzyme amount of 1 μmol of pyruvic acid generated by 1 g protein at 37°C for 15 minutes in the reaction system is defined as 1 unit.

$$\text{LDH activity (U/gprot)} = (\Delta A_{450} - b) \div a \times f \div C_{pr} \times 1000^*$$

[Note]

ΔA450: $OD_{Sample} - OD_{Control}$.

f: Dilution factor of sample before test.

Cpr: Concentration of protein in sample (gprot/L)

1000*: 1 L=1000 mL

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 (U/L)	26.00	154.00	287.00
%CV	2.3	2.2	2.1

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 (U/L)	26.00	154.00	287.00
%CV	6.8	7.3	7.5

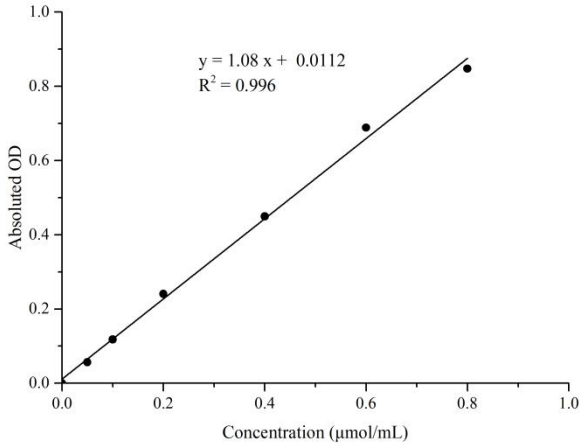
Sensitivity

The analytical sensitivity of the assay is 4 U/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 (μmol/mL)	0	0.05	0.1	0.2	0.4	0.6	0.8
Average OD	0.106	0.162	0.224	0.346	0.555	0.794	0.953
Absoluted OD	0.000	0.057	0.118	0.241	0.450	0.689	0.848



Appendix II Example Analysis

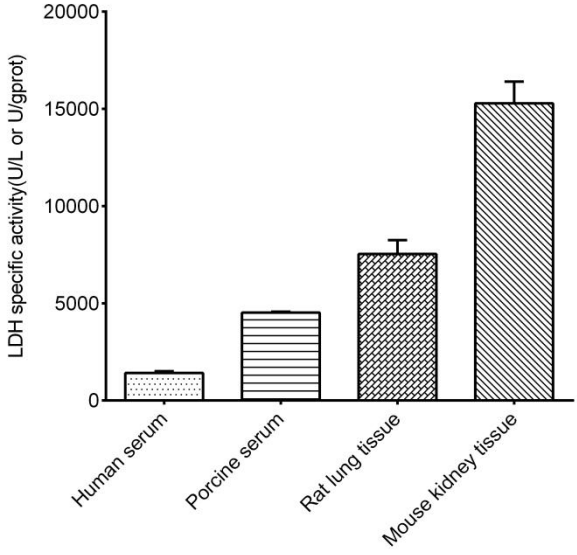
Example analysis：

For human serum, dilute human serum with PBS for 10 times, take 0.2 mL of diluted human serum and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 1.082 x + 0.0112, the average OD value of the sample is 0.308, the average OD value of the control is 0.143, and the calculation result is:

LDH activity (U/L) = (0.308 - 0.143 - 0.0112) ÷ 1.082 ×10 ×1000 = 1421.4 U/L

Detect human serum (dilute for 10 times), porcine serum (dilute for 15 times), 10% rat lung tissue homogenate (the concentration of protein is 3.97 gprot/L dilute for 500 times), and 10% mouse kidney tissue homogenate (the concentration of protein is 3.32 gprot/L dilute for 800 times) according to the protocol, the result is as follows：



Appendix III Publications

1. Liang L , Peng W , Qin A ,et al.Intracellularly Synthesized Artificial Exosome Treats Acute Lung Injury[J].ACS Nano, 2024, 18(32):15.DOI:10.1021/acsnano.4c01900.
2. Zhou Y , Du T , Yang C L ,et al.Extracellular vesicles encapsulated with caspase-1 inhibitor ameliorate experimental autoimmune myasthenia gravis through targeting macrophages[J].Journal of Controlled Release: Official Journal of the Controlled Release Society, 2023:364.DOI:10.1016/j.jconrel.2023.11.006.
3. Ma L ,Li Ma Li MaSchool of Design, Shanghai Jiao Tong University, Shanghai , People's Republic of ChinaR&D Center for Aromatic Plants, Shanghai Jiao Tong University, Shanghai , People's Republic of ChinaMore by Li Ma,,et al.Artemisia sieversiana Ehrhart ex Willd. Essential Oil and Its Main Component, Chamazulene: Their Photoprotective Effect against UVB-Induced Cellular Damage and Potential as Novel Natural Sunscreen Additives[J].ACS Sustainable Chemistry & Engineering, 2023(50):11.
4. Malla A , Gupta S , Sur R .Inhibition of lactate dehydrogenase A by diclofenac sodium induces apoptosis in HeLa cells through activation of AMPK[J].FEBS Journal, 2024, 291(16):25.DOI:10.1111/febs.17158.
5. Liu F , Lv L F , Bi F F ,et al.ITFG2 as a NEDD4-2 inhibitor: Preserving calcium homeostasis to prevent myocardial ischemic injury[J].Biochemical Pharmacology, 2024, 230.DOI:10.1016/j.bcp.2024.116597.

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.