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

Catalog No：E-BC-K046-M

Specification：96T(40 samples)/96T×5(232 samples)

Measuring instrument：Microplate reader（440-460 nm）

Detection range：6-500 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.

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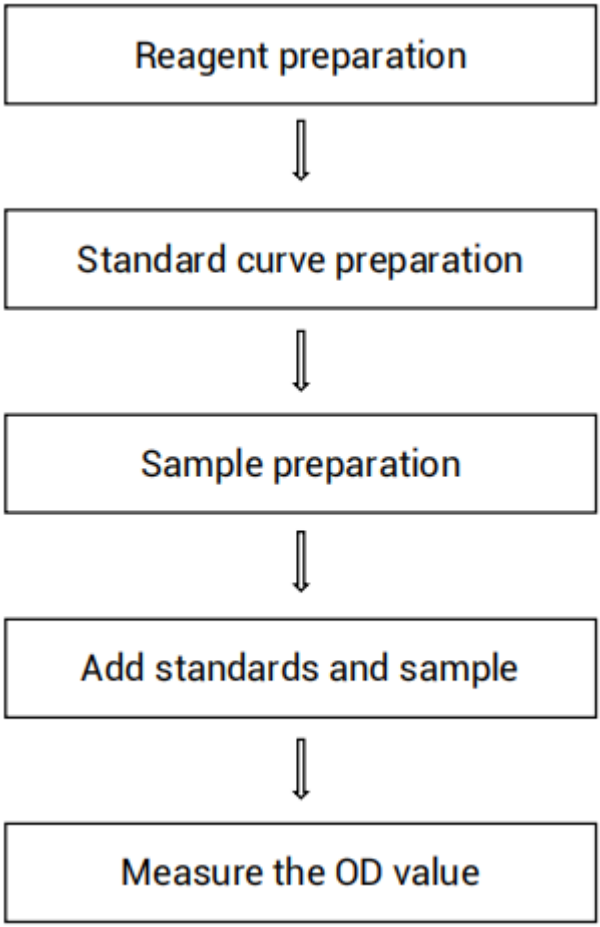
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Assay summary



Intended use

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

Detection principle

Using coenzyme I as a hydrogen carrier, LDH catalyze lactic acid to produce pyruvate. Pyruvate reacted with 2, 4-dinitrophenylhydrazine to form pyruvate dinitrophenylhydrazone, which was red-brown in alkaline solution, and the color depth was proportional to pyruvate concentration. The activity of LDH could be calculated by measuring OD value.

Kit components & storage

Item	Component	Size 1(96 T)	Size 2(96T×5)	Storage
Reagent 1	Substrate Buffer	5 mL × 1 vial	25 mL × 1 vial	2-8°C, 12 months
Reagent 2	Coenzyme I	Powder × 1 vial	Powder × 5 vials	2-8°C, 12 months
Reagent 3	Chromogenic Agent	5 mL × 1 vial	25 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 4	Alkali Reagent	5 mL × 1 vial	25 mL × 1 vial	2-8°C, 12 months
Reagent 5	2 μmol/mL Pyruvic Acid Standard	1 mL × 1 vial	5 mL × 1 vial	2-8°C, 12 months
	Microplate	96 wells	96 wells× 5	No requirement
	Plate Sealer	2 pieces	7 pieces	
	Sample Layout Sheet	1 piece	7 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(440-460 nm, optimum wavelength: 450 nm), Micropipettor, Multichannel pipette, Incubator

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 Preparation of coenzyme I application solution：

Dissolve one vial of Coenzyme I with 1.33 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 Preparation of alkali application solution：

For each well, prepare 200 μL of alkali application solution (mix well 20 μL of alkali reagent and 180 μL of double distilled water).It can be stored at 2–8 °C for up to 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, 1 μmol/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/mL)	**0**	**0.05**	**0.1**	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
2 μmol/mL standard (μL)	0	10	20	40	80	120	160	200
Double distilled water (μL)	400	390	380	360	320	280	240	200

Sample preparation

① Sample preparation

Sample requirements：

1. Avoid using hemolytic serum samples, as the LDH activity in red blood cells is about 100 times higher than that in serum.

2. SDS,Tween 20, NP-40, Triton X-100 and other detergents should not be included in the sample.

3. Oxalate anticoagulants should not be used because oxalate will inhibit the activity of LDH.

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 at 4°C 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 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 at 4°C 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
Human plasma	15-30
Porcine serum	10-20
Human hydrothorax	5-8
10% Mouse kidney tissue homogenization	500-800
10% Mouse lung tissue homogenization	500
10% Mouse liver tissue homogenization	500-800
HepG2 cells homogenization	100-300

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.

Operating steps

① Standard well: add 5 μL of double distilled water and 20 μL of standards with different concentrations into standard wells.
Sample well: add 20 μL of sample into sample wells.
Control well: add 5 μL of double distilled water and 20 μL of sample into control wells.
② Add 25 μL of substrate buffer to each well.
③ Add 5 μL of coenzyme I application solution to sample wells.
④ Mix fully and incubate at 37°C for 15 min.
⑤ Add 25 μL of chromogenic agent to each well. Mix fully and incubate at 37°C for 15 min.
⑥ Add 200 μL of reagent alkali application solution to each well.
⑦ Mix fully and stand at room temperature for 5 min. Measure the OD values of each well with microplate reader at 450 nm.

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)	14.50	250.00	662.00
%CV	2.3	1.6	1.5

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)	14.50	250.00	662.00
%CV	2.2	2.4	2.6

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 98%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/mL)	0.08	0.24	0.76
Observed Conc. (μmol/mL)	0.1	0.2	0.7
recovery rate(%)	98	99	97

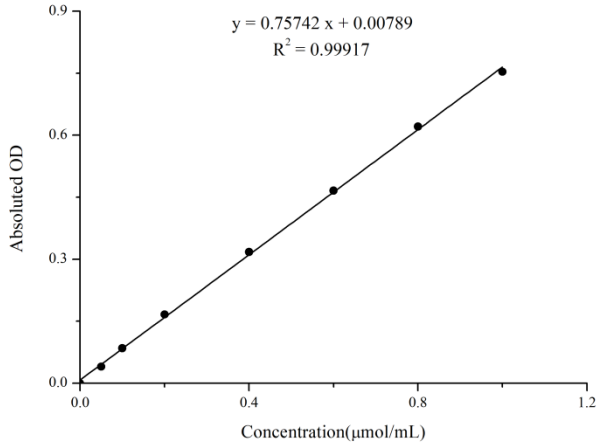
Sensitivity

The analytical sensitivity of the assay is 6 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**	**1**
Average OD	0.091	0.131	0.175	0.257	0.408	0.557	0.712	0.844
Absoluted OD	0.000	0.04	0.084	0.166	0.317	0.466	0.621	0.753



Appendix Π Example Analysis

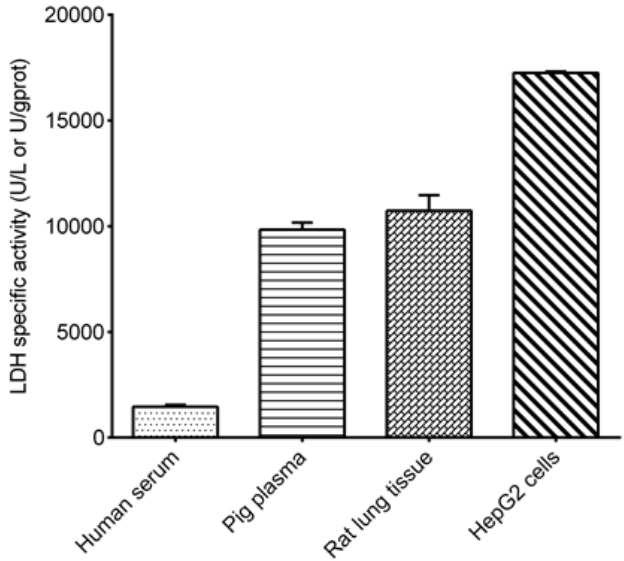
Example analysis：

For human serum, dilute human serum with PBS (0.01 M, pH 7.4) for 10 times, take 0.02 mL of diluted sample and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.75742 x + 0.00789, the average OD value of the sample is 0.232, the average OD value of the control is 0.115, and the result is:

LDH activity (U/L) = (0.232 - 0.115 - 0.00789) ÷ 0.75742 ×10 ×1000 = 1440.548 U/L

Detect human serum (dilute for 10 times), porcine plasma (dilute for 50 times), 10% rat lung tissue homogenate (the concentration of protein is 6.51 gprot/L, dilute for 500 times), HepG2 cells (the concentration of protein is 3.41 gprot/L, dilute for 20 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 , Wu Q , Li Y ,et al.Low-dose of polystyrene microplastics induce cardiotoxicity in mice and human-originated cardiac organoids[J].Environment International, 2023, 179.DOI:10.1016/j.envint.2023.108171.
3. 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.
4. Zhou Y , She R , Mei Z ,et al.Crosstalk between ferroptosis and necroptosis in cerebral ischemia/reperfusion injury and Naotaifang formula exerts neuroprotective effect via HSP90-GCN2-ATF4 pathway[J].Phytomedicine, 2024, 130(000):20.DOI:10.1016/j.phymed.2024.155399.
5. Hu K , Jiang X , Zhang J ,et al.Effect of Pulsatilla decoction on vulvovaginal candidiasis in mice. Evidences for its mechanisms of action[J].Phytomedicine, 2024, 128(000):11.DOI:10.1016/j.phymed.2024.155515.

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.