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

**Catalog No：E-BC-K1302-M**

**Specification：48T (48 samples)/96T (96 samples)**

**Measuring instrument：Microplate reader(340 nm)**

**Detection range：10-1935 U/L**

# **Elabscience**® Lactate Dehydrogenase (LDH) Activity Colorimetric Assay Kit (Lactate Substrate Method)

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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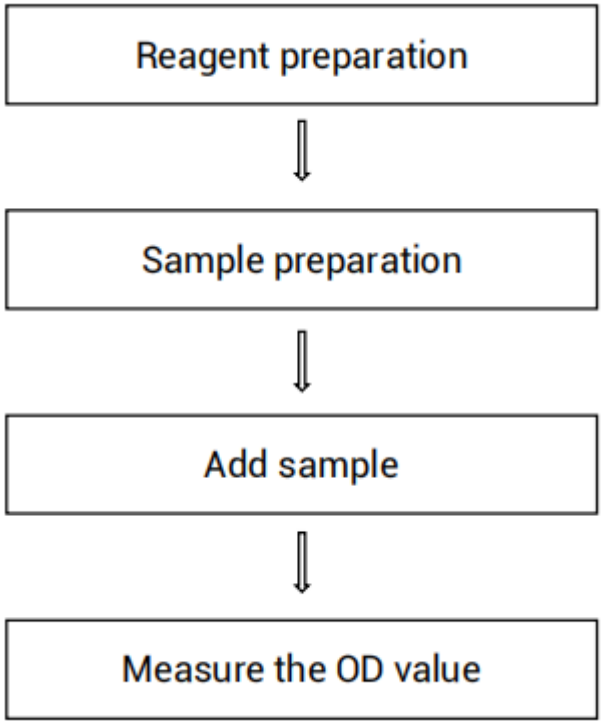
## Assay summary



## Intended use

This kit can be used to measure lactate dehydrogenase (LDH) activity in serum, plasma, animal tissue and cell samples.

## Detection principle

Lactate Dehydrogenase (LDH) is widely present in animal tissues, with the highest content in the kidneys, followed by the myocardium and bone muscle. It is one of the important enzyme systems for anaerobic glycolysis and gluconeogenesis. LDH catalyzes the generation of NADH from substrates. The activity of LDH can be characterized by measuring the rate of increase in absorbance at 340 nm.

## Kit components & storage

| Item | Component | Size 1(48 T) | Size 2(96 T) | Storage |
|---|---|---|---|---|
| Reagent 1 | Enzyme Buffer Solution | 11 mL × 1 vial | 22 mL × 1 vial | 2-8°C, 12 months, shading light |
| Reagent 2 | Enzyme Reaction Solution | 2.8 mL × 1 vial | 5.6 mL × 1 vial | 2-8°C, 12 months, shading light |
| | UV-Microplate | 96 wells | | No requirement |
| | Plate Sealer | 2 pieces | | |
| | Sample Layout Sheet | 1 piece | | |

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 (340 nm), Incubator, Vortex mixer, Homogenizer, Centrifuge

**Reagents:**

PBS(0.01 M，pH 7.4)

## Reagent preparation

Equilibrate all the reagents to 25°C before use.

## Sample preparation

**① Sample preparation**

**Serum or plasma samples:** detect directly.

**Tissue samples:**

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

② Homogenize 20 mg tissue in 180 μL PBS(0.01 M，pH 7.4) with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be used within 8 h.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

**Cell samples:**

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 1 mL PBS (0.01 M, pH 7.4) with a

ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10000 × g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be used within 8 h.

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 | 1 |
| Mouse serum | 1-2 |
| 10% Mouse liver tissue homogenate | 5-10 |
| 10% Mouse kidney tissue homogenate | 5-10 |
| 1×10^6 K562 Cells | 1 |

Note: The diluent is 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 Sample well: Add 10 μL of sample into wells.

② Add 200 μL of enzyme buffer solution into each well.

③ Mix fully and incubate at 37°C for 5 min.

④ Add 50 μL of enzyme reaction solution into each well.

⑤ Mix fully and incubate at 37°C for 1 min.

⑥ Measure the OD value of each well at 340 nm with microplate reader, as $A_1$.

⑦ Incubate at 37°C for 3 min. Measure the OD value of each well at 340 nm with microplate reader, as $A_2$, $\Delta A = A_2 - A_1$.

## Calculation

**The sample**:

**1. Serum or plasma samples:**

**Definition:** The amount of enzyme in 1 L serum or plasma per 1 min that produce 1 μmol of NADH at 37 °C is defined as 1 unit.

$$\text{LDH activity (U/L)} = \Delta A \times \frac{V_2}{\varepsilon \times d} \times 10\text{^}6^{*} \div T \div V_1 \times f = \Delta A \times 1935 \times f$$

**2. Tissue or cell samples:**

**Definition:** The amount of enzyme in 1 g tissue or cell protein per 1 min that produce 1 μmol of NADH at 37 °C is defined as 1 unit.

$$\text{LDH activity (U/gprot)} = \Delta A \times \frac{V_2}{\varepsilon \times d} \times 10\text{^}6^{*} \div T \div (C_{pr} \times V_1) \times f = \Delta A \times 1935 \div C_{pr} \times f$$

**3. Cell samples:**

**Definition:** The amount of enzyme in 1×10^6 of cells per 1 min that produce 1 μmol of NADH at 37 °C is defined as 1 unit.

$$\text{LDH activity (U/10\^6)} = \Delta A \times \frac{V_2}{\varepsilon \times d} \times 10\text{^}6^{*} \div T \div (n \times \frac{V_1}{V_3}) \times f = \Delta A \times 1.9 \div n \times f$$

**[Note]**

ΔA: $\Delta A = A_2 - A_1$.

ε: The molar extinction coefficient of at 340 nm， 6220 L/moL/cm.

d: Optical path, 0.72 cm.

10^6*： 1 mol = 1×10^6 μmol.

$V_1$: The volume of sample added to the reaction system, L.

$V_2$: The volume of reaction system, L.

$V_3$: The volume of PBS in the preparation step of sample, L.

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

n: The number of cell sample/10^6.

T: Reaction time, 3 min.

f: Dilution factor of sample before test.

# 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) | 226.0 | 556.0 | 1005.0 |
| %CV | 2.0 | 0.8 | 2.2 |

### 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 (U/L) | 62.0 | 148.0 | 292.0 |
| %CV | 8.2 | 5.4 | 2 |

### 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%.

| | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| Expected Conc. (U/L) | 119.0 | 523.0 | 988.0 |
| Observed Conc. (U/L) | 115.0 | 544.0 | 951.0 |
| Recovery rate (%) | 97 | 104 | 96 |

### Sensitivity

The analytical sensitivity of the assay is 10 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.

## Appendix Π Example Analysis

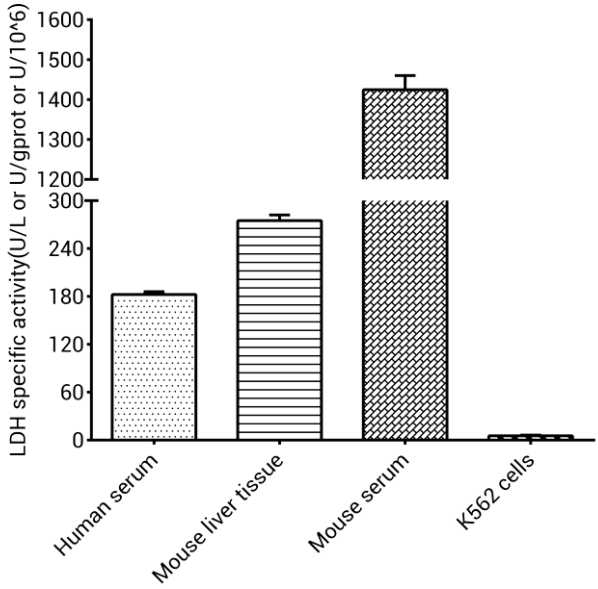
**Example analysis：**

Take 10 μL of 10% mouse liver tissue homogenate which dilute for 5 times and carry the assay according to the operation steps. The results are as follows:

The $A_1$ value of the sample well is 0.859, the $A_2$ value of the sample well is 1.275, the concentration of protein is 14.4 gprot/L. The calculation result is:

LDH activity (U/gprot) = (1.275 - 0.859) × 1935 ÷ 14.4 × 5 = 280 U/gprot

Detect human serum, 10% mouse liver tissue homogenate (the concentration of protein is 14.4 gprot/L, dilute for 5 times), mouse serum and 1×10^6 K562 cells, 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.