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

**Catalog No：E-BC-K766-M**

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

**Measuring instrument：Microplate reader（450 nm）**

**Detection range：0.11-39.9 U/L**

# **Elabscience**®Lactate Dehydrogenase (LDH) Activity Assay Kit (WST-8 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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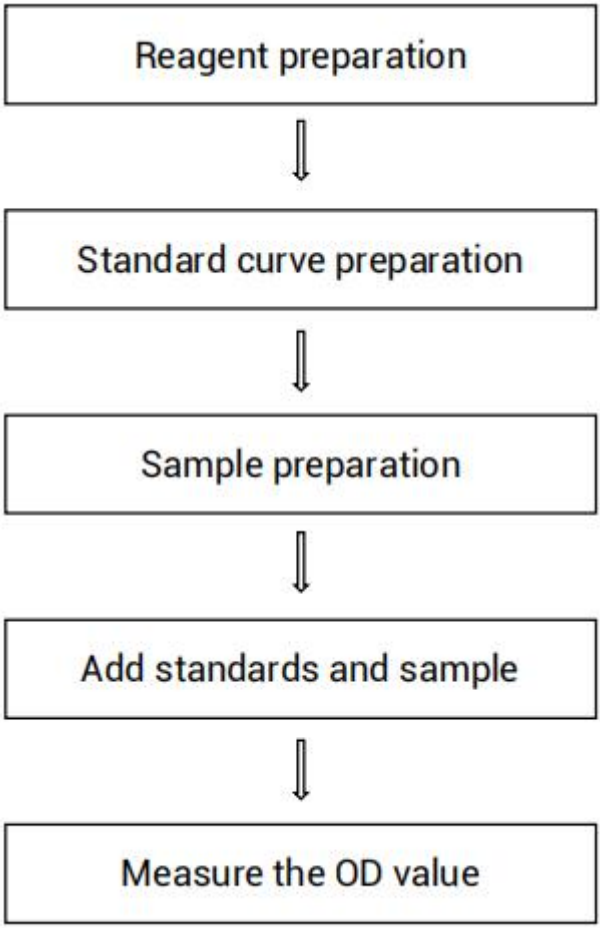
**Assay summary**



## Intended use

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

## Detection principle

Lactate dehydrogenase catalyzes the reaction of lactic acid with $NAD^+$ to produce pyruvic acid and NADH. NADH, under the action of PMS, transfer electrons to WST-8 to produce the yellow product, which has a characteristic absorption peak at 450 nm.

## Kit components & storage

| Item | Component | Size 1(48 T) | Size 2(96 T) | Storage |
|---|---|---|---|---|
| Reagent 1 | Lysis Solution | 60 mL × 1 vial | 60 mL × 2 vials | -20℃, 12 months |
| Reagent 2 | Substrate | 1.5 mL × 1 vial | 1.5 mL × 2 vials | -20℃, 12 months |
| Reagent 3 | Chromogenic Agent | 1.5 mL × 1 vial | 1.5 mL × 2 vials | -20℃, 12 months, shading light |
| Reagent 4 | Coenzyme | Powder × 1 vial | Powder × 1 vial | -20℃, 12 months |
| Reagent 5 | Stop Solution | 3 mL × 1 vial | 6 mL × 1 vial | -20℃, 12 months |
| Reagent 6 | NADH Standard | Powder × 1 vial | Powder × 1 vial | -20℃, 12 months |
| | Microplate | 48 wells | 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:**

Micropipettor, Vortex mixer, Incubator, Centrifuge, Microplate reader (450 nm)

**Reagents:**

Double distilled water, PBS (0.01 M, pH 7.4)

## Reagent preparation

① Preheat stop solution at 37°C for 20 min in advance and can be used only after it is completely clarified. Equilibrate other reagents to room temperature before use.

② Preparation of coenzyme working solution：
Dissolve one vial of coenzyme with 260 μL of double distilled water, mix well to dissolve. The coenzyme working solution should be prepared on spot. Store at -20°C for 7 days protected from light.

③ Preparation of reaction working solution：
For each well, prepare 50 μL of reaction working solution (mix well 24 μL of substrate, 24 μL of chromogenic agent and 2 μL of coenzyme working solution). The reaction working solution should be prepared on spot and stored protected from light.

④ Preparation of 5 mmol/L standard solution：
Dissolve one vial of NADH standard with 2 mL of double distilled water, mix well to dissolve. The 5 mmol/L standard solution should be prepared on spot. Store at -20°C for 7 days protected from light

⑤ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 5 mmol/L NADH standard with lysis solution to a serial

concentration. The recommended dilution gradient is as follows: 400, 300, 250, 200, 150, 100, 50, 0 μmol/L. Reference is as follows:

| Item | ① | ② | ③ | ④ | ⑤ | ⑥ | ⑦ | ⑧ |
|---|---|---|---|---|---|---|---|---|
| **Concentration (μmol/L)** | **0** | **50** | **100** | **150** | **200** | **250** | **300** | **400** |
| **5 mmol/L NADH standard (μL)** | 0 | 20 | 40 | 80 | 100 | 120 | 140 | 160 |
| **Lysis solution (μL)** | 1000 | 900 | 980 | 970 | 960 | 950 | 940 | 920 |

## 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.

**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 lysis solution with a dounce homogenizer at 4℃.

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) 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 Lyse 1×10^6 cells with 400 μL lysis solution. Place on the ice box and mix well every 5 min, lyse for 10 min.

④ Centrifuge at 15000×g for 10 min. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

**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.

**② Dilution of sample**

The recommended dilution factor for different samples is as follows (for reference only):

| Sample type | Dilution factor |
|---|---|
| Human serum | 10-20 |
| Dog serum | 10-20 |
| Mouse serum | 50-100 |
| Cynomolgus monkey serum | 10-20 |
| 10% Rat spleen tissue homogenate | 150-250 |
| 10% Rat liver tissue homogenate | 250-350 |
| 10% Rat kidney tissue homogenate | 250-350 |
| 10% Rat lung tissue homogenate | 250-350 |

Note: The diluent is lysis solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

## The key points of the assay

There should be no bubbles in the wells of the microplate when measuring the OD value.

## Operating steps

① Standard well: Take 50 μL of standard solution with different concentrations into the corresponding wells.
Sample well: Take 50 μL of sample into the corresponding wells.
② Add 50 μL of reaction working solution to each well.
③ Incubate at 37℃ for 10 min.
④ Add 50 μL of stop solution to each well.
⑤ Mix fully for 5 s with microplate reader. 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) , cell culture supernatant and other liquid sample:**

**Unit definition:** the enzyme amount of 1 μmol of NADH generated by 1 L of liquid sample per minute at 37℃ is defined as 1 unit.

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

**2. Tissue and cells sample:**

**Unit definition:** the enzyme amount of 1 μmol of NADH generated by 1 g tissue protein or cell protein per minute at 37℃ is defined as 1 unit.

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

**[Note]**

$\Delta A_{450}$: $OD_{Sample} - OD_{Blank}$.

f: Dilution factor of sample before test.

T: Reaction time (10 min).

$C_{pr}$: 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 (U/L)** | 2.60 | 18.50 | 26.00 |
| **%CV** | 2.6 | 2.3 | 2.0 |

### 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)** | 2.60 | 18.50 | 26.00 |
| **%CV** | 2.4 | 2.5 | 2.0 |

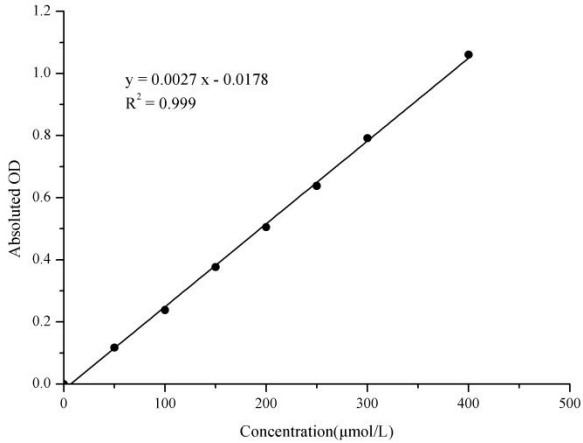
### Sensitivity

The analytical sensitivity of the assay is 0.11 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/L) | 0 | 50 | 100 | 150 | 200 | 250 | 300 | 400 |
|---|---|---|---|---|---|---|---|---|
| **Average OD** | 0.054 | 0.171 | 0.292 | 0.430 | 0.559 | 0.691 | 0.845 | 1.114 |
| **Absoluted OD** | 0.000 | 0.117 | 0.238 | 0.377 | 0.505 | 0.638 | 0.792 | 1.061 |



## Appendix Π Example Analysis

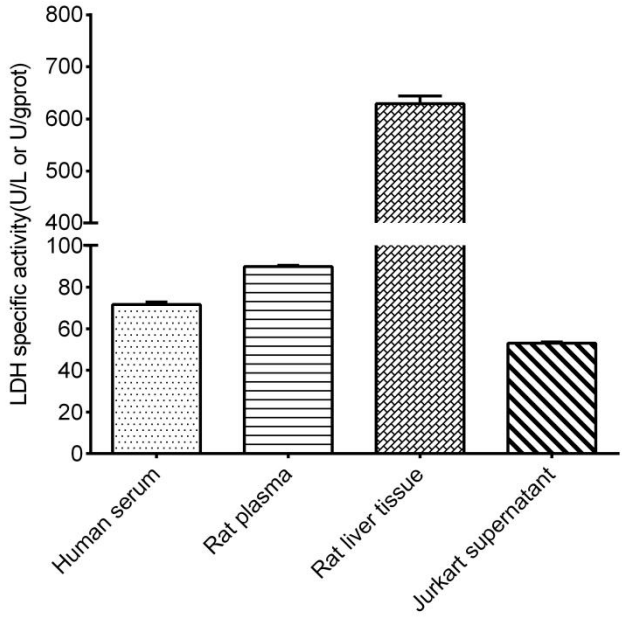
**Example analysis：**

Take 50 μL of human serum diluted for 10 times, and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 0.0027 x - 0.0178, the average OD value of the sample is 0.229, the average OD value of the blank is 0.054, and the calculation result is:

LDH activity (U/L) = ( 0.229 - 0.054 + 0.0178 ) ÷ 0.0027 ÷ 10 × 10 = 71.4 U/L

Detect human serum (dilute for 10 times), rat plasma (dilute for 10 times), 10% rat kidney tissue homogenate (the concentration of protein is 14.62 gprot/L dilute for 250 times) and Jurkart supernatant (dilute for 3 times) according to the protocol, the result is as follows：



# Appendix III Publications


1. Polcaro G, Liguori L, Manzo V, et al. rs822336 binding to C/EBPβ and NFIC modulates induction of PD-L1 expression and predicts anti-PD-1/PD-L1 therapy in advanced NSCLC[J]. Molecular Cancer, 2024, 23(1): 63.
2. 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.
3. Su P , Mao X , Ma J ,et al.ERRα promotes glycolytic metabolism and targets the NLRP3/caspase-1/GSDMD pathway to regulate pyroptosis in endometrial cancer[J].Journal of Experimental & Clinical Cancer Research (17569966), 2023, 42(1).DOI:10.1186/s13046-023-02834-7.
4. 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.
5. Teng Y, Yang Z, Peng Y, et al. Endoplasmic Reticulum Stress Nano - Orchestrators for Precisely Regulated Immunogenic Cell Death as Potent Cancer Vaccines[J]. Advanced Healthcare Materials, 2025, 14(1): 2401851.
6. 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.

## 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.