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

# D-Lactic Acid (D-LA) Assay Kit

Catalog No.: BC00002

Size: 50T/100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:


✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎Tel: 0086-27-87002838

Website: www.enkilife.com


**Shelf life:** Please refer to the label on the outer package.

**Techsupport:** In order to provide you with better service, please inform us the lot number on the label of the outer package.

## Basic Information

| | |
|---|---|
| **Product Name** | D-Lactic Acid (D-LA) Assay Kit |
| **Detection Method** | Colorimetric |
| **Sample Type** | Tissues, cells, serum, plasma |
| **Assay Type** | Quantitative |
| **Detection Instrument** | Microplate reader (450-490 nm, optimal detection wavelength 450 nm) |

## Product Introduction

Lactic acid plays a role in energy metabolism, pH regulation, and signal transmission in the human body. Lactic acid is effectively cleared by the body under normal circumstances, but in some cases, lactate levels may increase, leading to lactic acidosis. Lactic acidosis can be caused by a variety of reasons, including hypoxia, liver dysfunction, metabolic disorders, etc. In clinical practice, the degree of lactic acidosis can be assessed and treatment can be guided by measuring the lactate concentration in the blood.

## Product Features

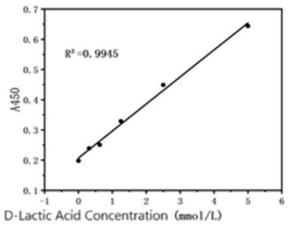
• Easy to use, high sensitivity and wide linear range.

## Principle

With oxidized coenzyme I ($NAD^+$) as hydrogen acceptor, lactate dehydrogenase (LDH) catalyzes the dehydrogenation of lactate to produce pyruvate, converting $NAD^+$ into reduced coenzyme I (NADH). Among them, N-methylphenazine methylsulfate (PMS) transfers hydrogen to reduce WST-8 to an orange coloring substance, the absorbance of which produces an absorption peak at a wavelength of 450nm, and the absorbance is linearly related to the lactic acid content.

The figure below shows the standard curve of D-lactic acid determination by this kit:

| D-Lactic Acid Concentration ( mmol/L ) | A450 |
|---|---|
| 5 | 0.644 |
| 2.5 | 0.449 |
| 1.25 | 0.329 |
| 0.625 | 0.251 |
| 0.3125 | 0.239 |
| 0 | 0.198 |




$y = 0.0894x + 0.2073$ ($R^2 = 0.9945$)

## Components

| No. | Components | Size (50T) | Size (100T) | Storage |
|---|---|---|---|---|
| Reagent 1 | Buffer | 10 mL | 20 mL | -20℃, store at 2-8℃ after opening. |
| Reagent 2 | Enzyme Stock Solution | 0.06 mL | 0.12 mL | -20℃, store at 2-8℃ after opening. |
| Reagent 3 | Color Developer | 1.2 mL | 1.2 mL/vial, 2 vials | -20℃, protect from light, store at 2-8℃ after opening. |
| Reagent 4 | Substrate B | 12 mL | 24 mL | -20℃, store at 2-8℃ after opening. |
| Reagent 5 | 10 mmol/L Standard | 1 mL | 1 mL/vial, 2 vials | -20℃, store at 2-8℃ after opening. |
| Consumable | Microplate | 1 plate | 1 plate | RT |
| Consumable | Plate Sealer | 2 pieces | 2 pieces | RT |

## Storage

The unopened kit can be stored at -20℃ for 12 months, and after opening, it can be stored at 2-8℃ for 6 months.

## Preparation

- **Sample handling**

1. Liquid samples such as serum and plasma: can be measured directly.
2. Tissue samples: routine homogenization (physiological saline (0.9% NaCl solution) or PBS (0.01 M, pH 7.4). After homogenization, centrifuge at 4 ℃, 10000×g for 10 min, take the supernatant and place it on ice for testing. Keep part of the supernatant for protein concentration determination.
3. Cell samples: Take $10^6$ cells and add 300 μL of physiological saline (0.9% NaCl solution) or PBS (0.01 M, pH 7.4) for homogenization. After homogenization, centrifuge at 4 ℃, 10000×g for 10 min, take the supernatant and place it on ice for testing. Keep part of the supernatant for protein concentration determination.

- **Preparation of the kit**

1. Before testing, reagents 1, 3, 4, and 5 must be equilibrated to room temperature, and reagent 2 must be placed on ice for later use.
2. Preparation of enzyme working solution: Mix at a volume ratio of Reagent 1: Reagent 2 = 100:1. Prepare immediately before use and use only on the same day.
3. Preparation of working solution: Mix enzyme working solution: reagent 3: reagent 4 at a volume ratio of 100:50:50. Prepare and use immediately. Prepare as needed and use within 12 hours.
4. Preparation of standard: Dilute the standard with reagent 1 by half dilution method to different concentrations such as 5, 2.5, 1.25, 0.625, 0.3125, 0 (blank well) mmol/L.

## Operation process

1. Standard wells: Take 10 μL of different concentrations of standards and add them to the corresponding standard wells; Sample wells: Take 10 μL of samples to be tested and add them to the sample wells.
2. Add 200 μL of the working solution to each standard and sample well.
3. Oscillate on a microplate reader for 5 s and incubate at 37°C for 10 min, measure the OD value of each well with an ELISA reader at 450 nm (450 nm-490 nm is acceptable, 450 nm is optimal).

**The operation table is as follows:**

| | **Standard Well** | **Measurement Well** |
|---|---|---|

| | | |
|---|---|---|
| Different concentrations of standard (μL) | 10 | -- |
| Sample to be tested (μL) | -- | 10 |
| Working solution (μL) | 200 | 200 |
| Shake on the microplate reader for 5 seconds, incubate at 37℃ for 10 minutes, and measure OD values at 450 nm. | | |

## Calculation

Standard fitting curve: y = ax + b

For serum (plasma), cell supernatant, and other liquid samples, the calculation formula for D-lactic acid content is:

D-Lactic acid content (mmol/L) = (ΔA450 - b) ÷ a × f

For tissue and cell samples, the calculation formula for D-lactic acid content is:

D-Lactic acid content (mmol/gprot) = (ΔA450 - b) ÷ a ÷ Cpr × f

**Annotation:**

ΔA 450: Sample measurement OD value - blank OD value (OD value when the standard concentration is 0)

f: dilution factor of the sample before adding it to the detection system

Cpr: protein concentration of the sample to be tested (gprot/L). When this kit detects tissue and cell samples, the total protein concentration needs to be measured. It is recommended to use the protein concentration determination kit (BCA method) (BC00006) produced by EnkiLife.

## Notes

1. Please read the manual carefully and adjust the instrument before the experiment, and strictly follow the manual for the experiment.
2. The detection range of the kit is not the same as the concentration range of the

substance to be measured in the sample. If the concentration of the substance to be measured in the sample is too high or too low, please dilute or concentrate the sample appropriately.

3. If the sample to be detected is not listed in the types of samples in the manual, it is recommended to perform a preliminary experiment to verify its detection effectiveness first.
4. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.