

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

L-Lactic Acid (L-LA) Assay Kit

Catalog No.: BC00004

Size: 50T

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	L-Lactic Acid (L-LA) Assay Kit
Detection Method	Colorimetric
Sample Type	Tissues, cells, serum, plasma
Assay Type	Quantitative
Sensitivity	0.0784mM
Range	0.15625-2.5mM
Detection Instrument	Microplate reader (450-490 nm, optimal detection wavelength 470 nm)

Product Introduction

Lactic acid (LA) is a hydroxy-containing carboxylic acid, with the chemical names 2-hydroxypropanoic acid, α -hydroxypropanoic acid, and propanol acid. Its molecular formula is $C_3H_6O_3$ and molecular weight is 90.08. Due to the asymmetry of its carbon atoms (i.e., chiral carbon), lactic acid usually exists in two optical isomer forms: dextrorotatory D-lactic acid (D(-)-Lactate) and levorotatory L-lactic acid (L(+)-Lactate). An equimolar mixture of the two forms yields racemic DL-lactic acid.

L-lactic acid is produced by the brain, red blood cells, skeletal muscles, and intestines, and metabolized by the liver, heart, and kidneys. Almost all lactic acid produced in the human body is L-lactic acid. L-lactic acid is mainly cleared from the blood by the liver, and hypoxia, acidosis, insufficient oxygen supply, and hypoperfusion can impair the liver's uptake of L-lactic acid. The concentration of L-lactic acid in the blood is crucial for diagnosing the condition of patients during intensive care and surgery. High concentrations of L-lactic acid can indicate diseases such as intestinal obstruction, cardiac arrest, and sepsis. The concentration of L-lactic acid in saliva can serve as an early diagnostic marker for cystic fibrosis, and L-lactic acid is also a biomarker for energy metabolism, as well as brain and tumor cells.

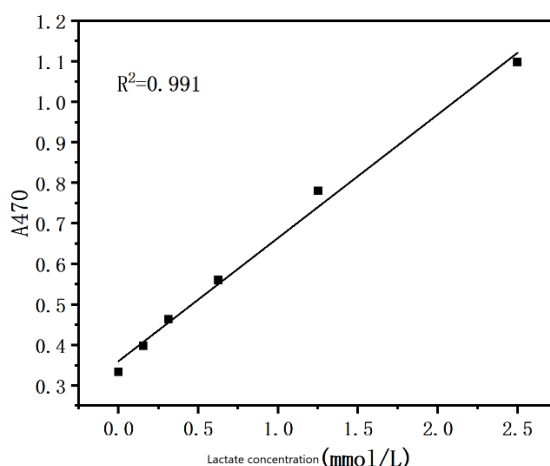
The L-Lactate Assay Kit with WST-8 is a detection kit based on the chromogenic reaction of WST-8. It enables rapid and highly sensitive determination of the contents of L-lactic acid and L-lactate in biological fluids such as serum or plasma, as well as in cell or tissue samples, via a colorimetric method.

Product Features

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

Principle

L-lactic acid is oxidized to pyruvate under the action of L-lactate dehydrogenase (L-LDH), during which nicotinamide adenine dinucleotide (NAD^+) is converted to its reduced form (NADH). The generated NADH first transfers electrons to phenazine methosulfate (PMS), reducing it to P-MHS. Subsequently, P-MHS transfers electrons to WST-8, reducing it to an orange-colored formazan. This formazan exhibits a maximum absorption peak at approximately 470 nm, and its absorbance is directly proportional to the concentration of L-lactic acid in the sample.



Components

No.	Components	Size (50T)	Storage
Reagent 1	Buffer	6 mL	-20°C, store at 2-8 °C after opening.
Reagent 2	Enzyme Stock Solution	0.06 mL	-20°C, store at 2-8 °C after opening.
Reagent 3	Color Developer	1.2 mL	-20°C, protect from light, store at 2-8°C after opening.
Reagent 4	Substrate B	1 mL	-20°C, store at 2-8°C after opening.
Reagent 5	2.5 mmol/L Standard	1 mL	-20°C, store at 2-8°C after opening.
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

The unopened kit can be stored at -20°C for 12 months, and after opening, it can be stored at 2-8°C 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 °C, 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 10⁶ 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 °C, 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, equilibrate all reagents to room temperature.
2. Preparation of enzyme working solution: Mix reagent 1 and reagent 2 at a volume ratio of 100:1. Prepare it before use and use it only on the same day.
3. Working solution preparation: Mix the enzyme working solution, Reagent 3, and Reagent 4 in a volume ratio of 100:20:20, prepare fresh and use as needed, use within 12 hours.
4. Preparation of standard: Dilute the standard into different concentrations such as 2.5, 1.25, 0.625, 0.3125, 0.15625, and 0 (blank well) mmol/L using reagent 1 according to the half-dilution method.

Operation process

1. Standard wells: Take 5 µL of different concentrations of standards and add them to the corresponding standard wells; Sample wells: Take 5 µL of samples to be tested and add them to the sample wells.
2. Add 140 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 at 470 nm (450nm-490nm is acceptable, 470nm is optimal) using an enzyme reader.

Calculation

Standard fitting curve: $y = ax + b$

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

$$\text{L-LA content (mmol/L)} = (\Delta A_{470} - b) \div a \times f$$

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

$$\text{L-LA content (mmol/gprot)} = (\Delta A_{470} - b) \div a \div \text{Cpr} \times f$$

Annotation:

ΔA_{470} : Sample measured 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 instructions carefully and adjust the instrument before the experiment, and conduct the experiment strictly in accordance with the instructions.
2. The detection range of the kit is not equivalent to the concentration range of the analyte in the sample. If the concentration of the analyte in the sample is too high or too low, please dilute or concentrate the sample appropriately.
3. If the sample being tested is not among the sample types listed in the instructions, it is recommended to conduct a preliminary experiment to verify the effectiveness of the test.
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.