Technical Manual

L-Lactic Acid (LA) Colorimetric Assay Kit

- Catalogue Code: MAES0056
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.12-7.0 mmol/L

Sensitivity:

0.10 mmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Lactic acid is an intermediate product of glucose metabolism in the body, which is mainly produced by red blood cells, striated muscle and brain tissue. The concentration of lactic acid in the blood mainly depends on the synthesis speed and metabolic rate of liver and kidney. The bidirectional conversion of lactic acid and pyruvate is regulated by lactate dehydrogenase (LDH).

3. Intended Use

This kit can be used to measure L-lactic acid (LA) content in serum (plasma), tissue, cells, culture supernatant samples.

4. Detection Principle

Using NAD^+ as H^+ receptor, LDH catalyzes the reaction of lactic acid and NAD^+ to generate pyruvic acid and NADH respectively. NBT is reduced to a kind of purple compound during the reaction. Measure the OD value at 530 nm, and the concentration of lactic acid can be calculated.

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	6 mL × 2 vials	2-8˚C, 6 months
Enzyme Stock Solution	0.06 mL × 2 vials	2-8˚C, 6 months
Chromogenic Agent	1.2 mL × 2 vials	2-8˚C, 6 months, away from direct sunlight
Stop Solution	12 mL × 2 vials	2-8˚C, 6 months
Standard (10 mmol/L)	1 mL × 2 vials	2-8˚C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (520-540 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

1. Severe hemolysis or jaundice may raise the OD value.

2. Avoid the formulation of bubbles when adding the liquid to the microplate.

7. Reagent preparation:

Preparation of **chromogenic agent:** Mix buffer solution, enzyme stock solution and chromogenic agent at the volume ratio of 100: 1: 20 fully. Prepare the fresh solution before use.

8. Sample Preparation

1. **Serum sample:**
   Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. **Plasma sample:**
   Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. **Cell sample:**
   Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) (μL) =1:300-500. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

4. **Tissue sample:**
   Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Homogenized Method:

Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), put the tissue pieces into a glass homogenized tube. Add homogenized medium into the homogenized tube and place the tube into an ice bath. Using a glass tamping rod, grind up and down for 6-8 min. Alternatively, place the tissue into a mortar and add liquid nitrogen to grind fully. Then, add the homogenized medium.

Mechanical Homogenate: Weigh the tissue in an EP tube. Add the homogenized medium to homogenize the tissue with an homogenizer instrument (60 Hz, 90s) in an ice bath. (For skin, muscle and plant tissue samples, prolong homogenization time accordingly). **Ultrasonication:** Treat the cells with an ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, total time is 5 min).

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.12-7.0 mmol/L).

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

Sample Type:	Dilution Factor:
Human serum	2-5
10% Rat kidney tissue homogenate	1-3
10% Rat brain tissue homogenate	1
HepG2 cell culture supernatant	1
HepG2 cells	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 530 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps

The preparation of standard curve

Dilute 10 mmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 3, 4, 5, 6, 7 mmol/L.

The measurement of samples

1. **Standard well:** Add 5 µL of standard solution with different concentrations to the wells.
   Sample well: Add 5 µL of sample to the wells.

2. Add 120 µL of chromogenic agent to each well.

3. Mix fully and incubate at 37℃ for 5 min

4. Add 180 µL of stop solution to each well.

5. Mix fully for 5 s with microplate reader. Measure the OD values of each well at 530 nm with microplate reader.

Operation Table

	Standard well	Sample well
Standards with different concentrations (µL)	5	
Sample (µL)		5
Chromogenic reagent (µL)	120	120
Mix fully and incubate at 37℃ for 5 min.		
Stop solution (µL)	180	180
Mix fully for 5 s with microplate reader. Measure the OD values of each well at 530 nm with microplate reader.		

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Serum (plasma), culture supernatant and other liquid sample:

$$\text{LA content (mmol/L)} = (\Delta A_{530} - b) \div a \times f$$

2. Tissue and cells sample:

$$\text{LA content (mmol/gprot)} = (\Delta A_{530} - b) \div a \div C_{pr} \times f$$

y: The absolute OD value of standard
x: The concentration of standard.
a: The slope of standard curve .
b: The intercept of standard curve.
ΔA_{530}: Absolute OD ($OD_{Sample} - OD_{Blank}$)
f: Dilution factor of sample before test.
C_{pr}: Concentration of protein in sample, gprot/L.

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12. Performance Characteristics

Detection Range	0.12-7.0 mmol/L
Sensitivity	0.10 mmol/L
Average recovery rate (%)	105
Average inter-assay CV (%)	3.5
Average intra-assay CV (%)	1.4

Analysis

Dilute the human serum with double distilled water for 5 times, take 5 μL of diluted sample, carry the assay according to the operation table.

The results are as follows:

Standard curve: y = 0.12763 x – 0.01033, the average OD value of the sample is 0.414, the average OD value of the blank well is 0.052, and the calculation result is:

$$\boldsymbol{LA\ content}\left(\frac{\boldsymbol{mmol}}{\boldsymbol{L}}\right) = (\mathbf{0.414} - \mathbf{0.052} + \mathbf{0.01033}) \div \mathbf{0.12763} \times \mathbf{5}$$

$$= \mathbf{14.59}\ \boldsymbol{mmol/L}$$

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

Notes:

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