Technical Manual

D-Lactic Acid (LA) Colorimetric Assay Kit

- Catalogue Code: MAES0015
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.06-8.0 mmol/L

Sensitivity:

0.06 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

D-Lactic acid is an isomer of L-lactic acid. D-lactic acid is a product of bacterial metabolism. Due to the metabolism of pyruvaldehyde, D-lactic acid is usually present in the blood of mammals. The increase in D-lactic acid concentration may be due to the production by excessive gastrointestinal microbial. Subclinical elevation of D-lactic acid is an indicator of sepsis and trauma. The accumulation of D-lactic acid can lead to D-lactic acidosis, which is manifested as unlocalized nervous system symptoms and encephalopathy symptoms.

3. Intended Use

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

4. Detection Principle

Using NAD^+ as H^+ receptor, D-lactate dehydrogenase (LDH) catalyzes the reaction of D-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 D-lactic acid can be calculated.

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	12 mL × 1 vial	2-8°C, 6 months
Enzyme Stock Solution	0.12 mL × 1 vial	2-8°C, 6 months
Chromogenic Agent	1.2 mL × 2 vials	2-8°C, 6 months, avoid direct sunlight
Stop Solution	24 mL × 1 vial	2-8°C, 6 months
Standard Solution (10 mmol/L)	2.0 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (530 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

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

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

3. If the D-lactic acid content is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (E-BC-K318-M).

7. Reagent Preparation

1. Bring buffer solution, chromogenic agent, stop solution and standard solution (10 mmol/L) to room temperature before use. Preserve enzyme stock solution on ice for use.

2. Preparation of **enzyme working solution:** Mix buffer solution and enzyme stock solution at the volume ratio of 100: 1 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. 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 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.

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 (0.06-8.0 mmol/L).

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

Sample Type:	Dilution Factor
Human plasma	1
Human serum	1
Rat serum	1
Rat plasma	1
Mouse serum	1
Rabbit serum	1
10% Rat kidney tissue homogenate	2-3
10% Mouse brain tissue homogenate	1

Note: The diluent of is 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 standard solution (10 mmol/L) with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 1.0, 2.0, 4.0, 5.0, 6.0, 7.0, 8.0 mmol/L.

The measurement of samples

1. **Standard well:** add 5 µL of standards with different concentrations into the standard wells.
   Sample well: add 5 µL of sample into the sample wells.

2. Add 100 µL of enzyme working solution to each well.

3. Add 20 µL of chromogenic agent to each well.

4. Mix fully and incubate at 37°C for 10 min.

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

6. 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
Standard of different concentrations (μL)	5	
Sample (μL)		5
Enzyme working solution (μL)	100	100
Chromogenic agent (μL)	20	20
Mix fully and incubate at 37°C for 10 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 fluorescence value (F) 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 F value of sample. The standard curve is: y= ax + b.

1. Serum (plasma) sample:

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

2. Tissue sample:

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

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the change OD value when the standard concentration is 0).

x: The concentration of Standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{530}: $OD_{Sample} - OD_{Blank}$

f: Dilution factor of sample before tested

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

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12. Performance Characteristics

Detection Range	0.06-8.0 mmol/L
Sensitivity	0.06 mmol/L
Average recovery rate (%)	99
Average inter-assay CV (%)	7.7
Average intra-assay CV (%)	3.8

Analysis

For human serum, take 5 μL of human serum, carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.1082 x + 0.0232, the average OD value of the sample is 0.307, the average OD value of the blank is 0.148, and the calculation result is:

D-LA content (mmol/L) = (0.307- 0.148 – 0.0232) ÷ 0.1082
= 1.26 mmol/L

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent 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.

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