D-Lactic Acid/Lactate Colorimetric Assay Kit

Catalog No: E-BC-K002-M

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.06 mmol/L

Detection range: 0.06-8.0 mmol/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲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.

▲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.

Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	12 mL × 1 vial	2-8°C , 6 months
Reagent 2	Enzyme Stock Solution	0.12 mL × 1 vial	2-8°C , 6 months
Reagent 3	Chromogenic Agent	1.2 mL × 2 vials	2-8°C , 6 months, shading light
Reagent 4	Stop Solution	24 mL × 1 vial	2-8°C , 6 months
Reagent 5	10 mmol/L Standard Solution	2.0 mL × 1 vial	2-8°C , 6 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

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.

Materials prepared by users

Instruments

Micropipettor, Vortex mixer, Incubator, Centrifuge, Microplate reader (530 nm)

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents

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

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

▲Precautions

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

▲The key points of the assay

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).

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 2 working solution:
   Mix reagent 1 and reagent 2 at the volume ratio of 100: 1 fully. Prepare the fresh solution before use.
2. Bring reagent 1, reagent 3, reagent 4 and reagent 5 to room temperature before use. Preserve reagent 2 on ice for use.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and 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 is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	530 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲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.

▲Operating steps

1. The preparation of standard curve

Dilute 10 mmol/L standard solution 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.

2. 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 reagent 2 working solution to each well.

3) Add 20 μL of reagent 3 to each well.

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

5) Add 180 μL of reagent 4 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
Standards with different concentrations (μL)	5	
Sample (μL)		5
Reagent 2 working solution (μL)	100	100
Reagent 3 (μL)	20	20
Mix fully and incubate at 37°C for 10 min.		
Reagent 4 (μL)	180	180
Mix fully for 5 s with microplate reader. Measure the OD values of each well at 530 nm with microplate reader.		

▲Calculation

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) sample:

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

2. Tissue sample:

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

Note:

y: $OD_{Standard} - OD_{Blank}$. ($OD_{Blank}$ is the 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 test.

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

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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

▲Example analysis

For human serum, take 5 μL of human serum and 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:

$$\text{D-LA content (mmol/L)} = \frac{(0.307 - 0.148 - 0.0232)}{0.1082} = 1.26 \text{ mmol/L}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge 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.

▲ Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g 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.

▲ 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 homogenized medium (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. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Note:

1. Homogenized medium: Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).
2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

▲ Note for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.