TECHNICAL MANUAL

D-Lactic Acid/Lactate Colorimetric Assay Kit

- **SKU CODE:** MAES0015
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Standard curve preparation
- Sample preparation
- Add standards and samples
- Add enzyme working solution
- Add chromogenic agent and incubate
- Add stop solution and measure OD values

2. Intended use

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

3. 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 purple compound during the reaction. The OD value is measured at 530 nm, and the concentration of D-lactic acid can be calculated.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	6 mL × 1 vial	12 mL × 1 vial	2-8°C, 12 months
Reagent 2	Enzyme Stock Solution	0.06 mL × 1 vial	0.12 mL × 1 vial	2-8°C, 12 months
Reagent 3	Chromogenic Agent	1.2 mL × 1 vial	1.2 mL × 2 vials	2-8°C, 12 months, protect from light
Reagent 4	Stop Solution	12 mL × 1 vial	24 mL × 1 vial	2-8°C, 12 months
Reagent 5	10 mmol/L Standard Solution	1.0 mL × 1 vial	2.0 mL × 1 vial	2-8°C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments: Micropipettor, vortex mixer, incubator, centrifuge, microplate reader (520-550 nm, optimal wavelength: 530 nm)

6. Reagent preparation

1. Keep enzyme stock solution on ice for use. Equilibrate all reagents to room temperature before use.
2. **Preparation of enzyme working solution:** Before testing, prepare sufficient enzyme working solution according to the number of test wells. For example, to prepare 505 µL of enzyme working solution, thoroughly mix 5 µL of enzyme stock solution with 500 µL of buffer solution. The enzyme working solution should be prepared fresh.
3. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 10 mmol/L standard solution with double distilled water to serial concentrations. The recommended dilution gradient is: 0, 1, 2, 4, 5, 6, 7, 8 mmol/L.

7. Sample preparation

Serum and plasma: Detect directly. If not tested on the same day, serum or plasma can be stored at -80°C for one month.

Saliva: Rinse mouth with clear water, collect saliva 30 minutes later, centrifuge at 10,000 x g for 10 min at 4°C. Take the supernatant and preserve on ice for detection.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation: 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 20 mg tissue in 180 µL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

4. Centrifuge at 10,000 x g for 10 min to remove insoluble material. Collect supernatant and keep on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant.

Dilution of sample: The recommended dilution factor for different samples is as follows (for reference only):

- 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). For dilution of other sample types, perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. Severe hemolysis or jaundice may increase the OD value.
2. Prevent bubble formation when adding liquid to the microplate.
3. If D-lactic acid content is calculated by protein concentration, the protein concentration of the sample needs to be determined separately.

9. Operating steps

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 thoroughly and incubate at 37°C for 10 min.
5. Add 180 μL of stop solution to each well.
6. Mix thoroughly for 5 seconds with microplate reader. Measure the OD values of each well at 530 nm with microplate reader.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.

3. Plot the standard curve using absolute OD value of standards and corresponding concentrations as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

1. Serum (plasma) sample:

D-LA content (mmol/L) = (ΔA_530 - b) ÷ a × f

2. Tissue sample:

D-LA content (mmol/g_{prot}) = (ΔA_530 - b) ÷ a × f ÷ C_{pr}

[Note]

ΔA_530: OD_{Sample} - OD_{Blank}

f: Dilution factor of sample before test

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

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.50	2.50	5.00
%CV	4.2	3.6	3.6

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.50	2.50	5.00
%CV	8.2	7.5	7.4

Recovery

Standard 1	Standard 2	Standard 3
1.5	4.5	6.5
1.5	4.4	6.4
101	98	98

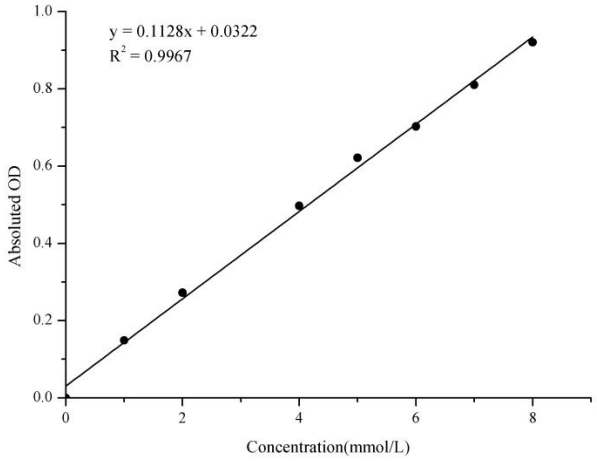
Sensitivity

The analytical sensitivity of the assay is 0.06 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve data

Concentration (mmol/L)	OD value 1	OD value 2	Average OD	Absolute OD
0	0.138	0.136	0.137	0.000
1.0	0.284	0.288	0.286	0.149
2.0	0.414	0.405	0.410	0.273
4.0	0.648	0.620	0.634	0.497
5.0	0.752	0.765	0.759	0.622
6.0	0.856	0.823	0.840	0.703
7.0	0.934	0.961	0.948	0.811
8.0	1.083	1.032	1.058	0.921

11. Appendix II Example Analysis



Example analysis:

For human serum, take 5 µL of human serum and perform the assay according to the operation protocol. The results are as follows:

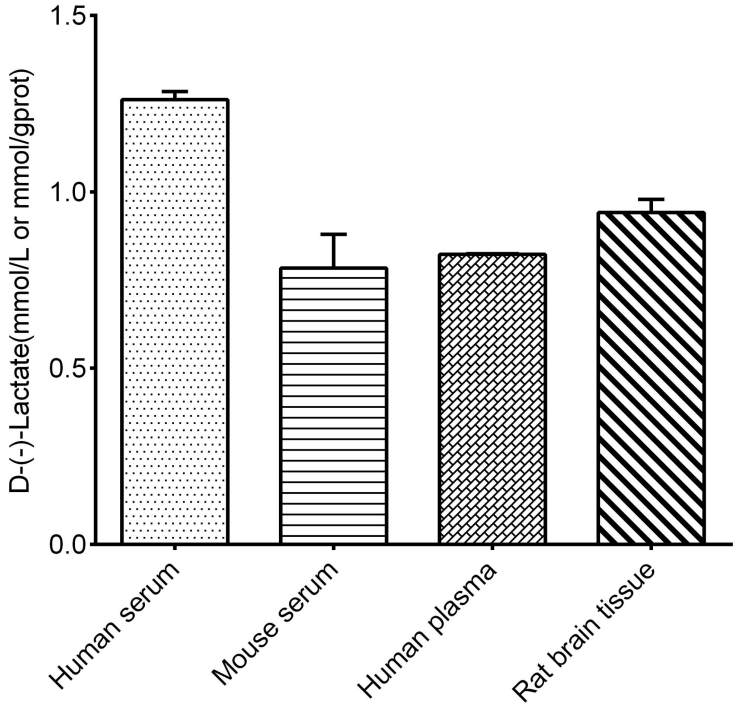
Standard curve: y = 0.1082x + 0.0232

Average OD value of the sample: 0.307

Average OD value of the blank: 0.148

Calculation result:

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



Detect human serum, mouse serum, human plasma, and 10% rat brain tissue homogenate (protein concentration: 3.64 g_{prot}/L) according to the protocol.

12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance is not within the detection range exactly, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. The experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

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Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.