TECHNICAL MANUAL

L-Lactic Acid/Lactate (LA) Colorimetric Assay Kit

- **SKU CODE:** MAES0056
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Intended use

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

2. Detection principle

Lactate dehydrogenase (LDH) catalyzes the conversion of lactate to pyruvate, which reduces NAD+ to NADH. In this process, PMS acts as a hydrogen carrier, facilitating the reduction of NBT to a purple product. The OD value is measured at 530 nm, and the concentration of lactic acid can be calculated.

3. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3(500 Assays)	Storage
Reagent 1	Buffer Solution	6 mL × 1 vial	6 mL × 2 vials	60 mL × 1 vial	2-8 °C, 12 months
Reagent 2	Enzyme Stock Solution	0.6 mL × 1 vial	1.2 mL × 1 vial	3 mL × 2 vials	2-8 °C, 12 months
Reagent 3	Chromogenic Agent	1.2 mL × 1 vial	1.2 mL × 2 vials	12 mL × 1 vial	2-8 °C, 12 months, shading light
Reagent 4	Stop Solution	12 mL × 1 vial	12 mL × 2 vials	60 mL × 2 vials	2-8 °C, 12 months
Reagent 5	10 mmol/L Lactic Acid Standard	1 mL × 1 vial	1 mL × 2 vials	10 mL × 1 vial	2-8 °C, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

4. Materials prepared by users

Instruments:

Microplate reader (520-540 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents from different kits cannot be mixed with each other. For small volumes of reagents, please centrifuge before use to ensure sufficient reagent is obtained.

5. Reagent preparation

1. Keep enzyme stock solution on ice during use. Equilibrate other 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, prepare 110 µL of enzyme working solution by mixing 100 µL of buffer solution with 10 µL of enzyme stock solution. Mix well. The enzyme working solution should be prepared immediately before use.
3. **Preparation of standard curve::** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 10 mmol/L standard with deionized water to create a serial concentration. The recommended dilution gradient is: 0, 1, 2, 3, 4, 5, 6, 7 mmol/L.

6. Sample preparation

Sample preparation:

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

Cell culture supernatant: Collect fresh cell culture supernatant and centrifuge at 10,000×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×g for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Cells:

1. Harvest the number of cells needed for each assay (initial recommendation: 3×10^6-5×10^6 cells).

2. Wash cells with PBS (0.01 M, pH 7.4).

3. Homogenize 3×10^6-5×10^6 cells in 200 µL PBS (0.01 M, pH 7.4) with an ultrasonic cell disruptor at 4 °C.

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

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

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

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

7. The key points of the assay

1. Aliquot buffer solution according to the required volume before use to avoid contamination.
2. Add the sample by touching the bottom of the well.

8. Operating steps

1. Standard well: Add 5 µL of standards with different concentrations to the corresponding wells. Sample well: Add 5 µL of sample to the corresponding wells.
2. Add 100 µL of enzyme working solution to each well.
3. Add 20 µL of chromogenic agent to each well.
4. Incubate at 37 °C for 10 min.

5. Add 180 µL of stop solution to each well.
6. Mix well for 5 s with microplate reader. Measure the OD values of each well at 530 nm with microplate reader.

9. Calculation

The standard curve:

1. Average the duplicate readings 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 the absolute OD value of standards and corresponding concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

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

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

2. Tissue and cell samples:

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

[Note]

ΔA_530: Absolute OD (OD_{Sample} - OD_{Blank}).

f: Dilution factor of sample before test.

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

10. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.50	2.10	4.60

Parameters	Sample 1	Sample 2	Sample 3
%CV	1.8	1.2	1.2

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.50	2.10	4.60
%CV	3.1	3.4	4.0

Recovery

Three samples of high, middle, and low concentration were tested with 6 parallel measurements for each concentration to obtain an average recovery rate of 105%.

Recovery

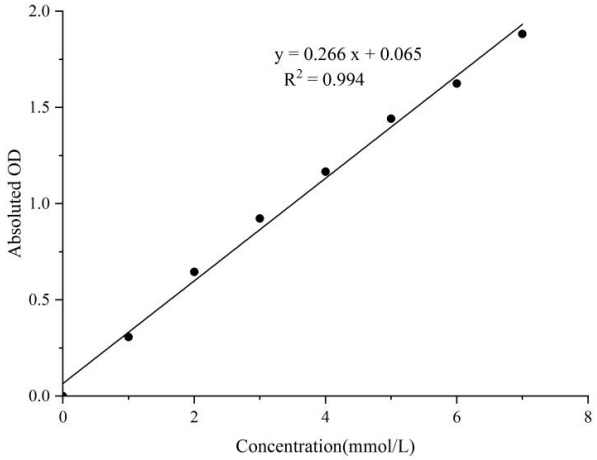
Standard 1	Standard 2	Standard 3
1.5	3.6	5.4
1.6	3.7	5.7
106	103	106

Sensitivity

The analytical sensitivity of the assay is 0.12 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

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g., operator, pipetting technique, or temperature effects), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (mmol/L)	0	1	2	3	4	5	6	7
Average OD	0.160	0.466	0.805	1.082	1.327	1.601	1.784	2.042
Absolute OD	0.000	0.307	0.646	0.923	1.167	1.442	1.624	1.882

11. Appendix II Example Analysis

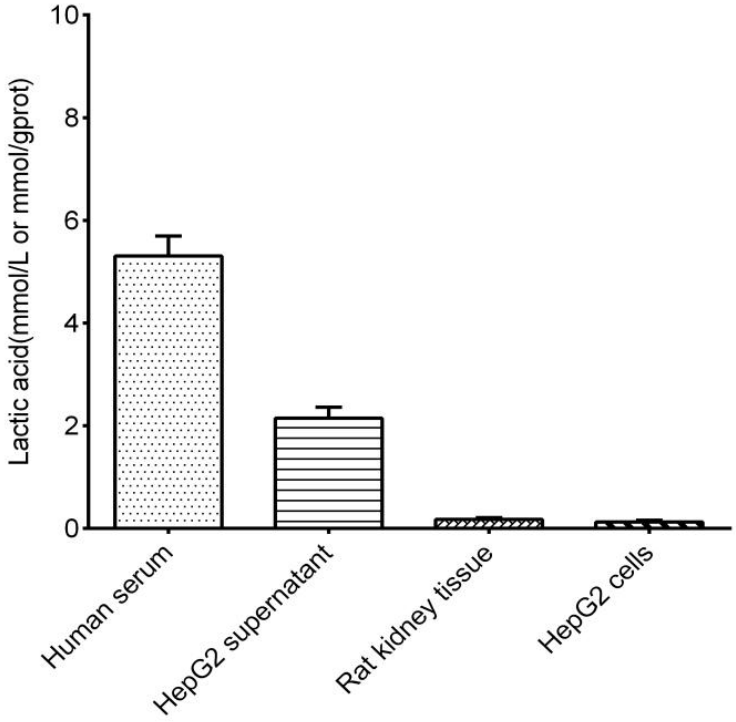
Example analysis:

Dilute human serum with double distilled water 5-fold, take 5 µL of diluted sample and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.266x + 0.065, 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:

LA content (mmol/L) = (0.414 - 0.052 - 0.065) ÷ 0.266 × 5 = 5.58 mmol/L

Detect human serum (diluted 5-fold), HepG2 cell culture supernatant, 10% rat kidney tissue homogenate (protein concentration: 7.18 gprot/L), and HepG2 cells (protein concentration: 9.55 gprot/L) according to the protocol. The results are as follows:



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 falls outside the detection range, 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. 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.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

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