TECHNICAL MANUAL

Lactate Dehydrogenase (LDH) Activity Assay Kit

- **SKU CODE:** MAES0058
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate at 37°C for 15 minutes
- Add chromogenic agent
- Incubate at 37°C for 15 minutes
- Add alkali reagent
- Measure OD at 450 nm

2. Intended use

This kit can be used to measure lactate dehydrogenase (LDH) activity in tissues, serum (plasma), cultured cells, pleural effusion, and other samples.

3. Detection principle

Using coenzyme I as a hydrogen carrier, LDH catalyzes the conversion of lactic acid to produce pyruvate. Pyruvate reacts with 2,4-dinitrophenylhydrazine to form pyruvate dinitrophenylhydrazone, which appears red-brown in alkaline solution. The color intensity is proportional to pyruvate concentration. The activity of LDH can be calculated by measuring the OD value.

4. Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	Substrate Buffer	5 mL x 1 vial	25 mL x 1 vial	2-8°C, 12 months
Reagent 2	Coenzyme I	Powder x 1 vial	Powder x 5 vials	2-8°C, 12 months
Reagent 3	Chromogenic Agent	5 mL x 1 vial	25 mL x 1 vial	2-8°C, 12 months, protect from light
Reagent 4	Alkali Reagent	5 mL x 1 vial	25 mL x 1 vial	2-8°C, 12 months

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 5	2 μmol/mL Pyruvic Acid Standard	1 mL x 1 vial	5 mL x 1 vial	2-8°C, 12 months
	Microplate	96 wells	/	No requirement
	Plate Sealer	2 pieces		

5. Materials prepared by users

Instruments:

Microplate reader (440-460 nm, optimum wavelength: 450 nm), Micropipettor, Multichannel pipette, Incubator

Reagents:

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

6. Reagent preparation

1. Keep Coenzyme I on ice during use. Equilibrate other reagents to room temperature before use.
2. **Preparation of coenzyme I application solution::** Dissolve one vial of Coenzyme I with 1.33 mL of double distilled water. Mix well to dissolve. Store at 2-8°C for 15 days or in aliquots at -20°C for 1 month.
3. **Preparation of alkali application solution::** For each well, prepare 200 μL of alkali application solution by mixing 20 μL of alkali reagent with 180 μL of double distilled water. It can be stored at 2-8°C for up to 7 days.
4. **Preparation of standard curve::** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 2 μmol/mL pyruvic acid standard solution with double distilled water to serial concentrations. The recommended dilution gradient is as follows: 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1 μmol/mL.

7. Sample preparation

Sample requirements:

1. Avoid using hemolytic serum samples, as LDH activity in red blood cells is approximately 100 times higher than in serum.

2. SDS, Tween 20, NP-40, Triton X-100, and other detergents should not be present in the sample.

3. Oxalate anticoagulants should not be used because oxalate inhibits LDH activity.

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

Tissue sample:

1. Harvest the required tissue amount 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 normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) using a dounce homogenizer at 4°C.

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

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

Cell (adherent or suspension) sample:

1. Harvest the required number of cells for each assay (initial recommendation: 1×10^6 cells).

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

3. Homogenize 1×10^6 cells in 300 µL normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) using an ultrasonic cell disruptor at 4°C.

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

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

Dilution of sample:

The recommended dilution factors for different samples are as follows (for reference only):

- Human serum: 10-fold
- Human plasma: 15-30-fold
- Porcine serum: 10-20-fold
- Human pleural effusion: 5-8-fold
- 10% Mouse kidney tissue homogenate: 500-800-fold
- 10% Mouse lung tissue homogenate: 500-fold
- 10% Mouse liver tissue homogenate: 500-800-fold
- HepG2 cell homogenate: 100-300-fold

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For other sample types, please perform a pretest to determine the appropriate dilution factor.

8. Operating steps

1. Standard well: Add 5 μL of double distilled water and 20 μL of standards with different concentrations to standard wells. Sample well: Add 20 μL of sample to sample wells. Control well: Add 5 μL of double distilled water and 20 μL of sample to control wells.
2. Add 25 μL of substrate buffer to each well.
3. Add 5 μL of coenzyme I application solution to sample wells.
4. Mix thoroughly and incubate at 37°C for 15 minutes.
5. Add 25 μL of chromogenic agent to each well. Mix thoroughly and incubate at 37°C for 15 minutes.
6. Add 200 μL of alkali application solution to each well.
7. Mix thoroughly and stand at room temperature for 5 minutes. Measure the OD values of each well using a microplate reader at 450 nm.

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 as the y-axis and corresponding concentration as the x-axis. Create the standard curve (y = ax + b) using graphing software (or Excel).

The sample:

1. Serum (plasma) sample:

Unit definition: The enzyme amount that generates 1 μmol of pyruvic acid per 1 L of sample at 37°C for 15 minutes in the reaction system is defined as 1 unit.

LDH activity (U/L) = (ΔA_450 - b) ÷ a × f × 1000*

2. Tissue and cell sample:

Unit definition: The enzyme amount that generates 1 μmol of pyruvic acid per 1 g protein at 37°C for 15 minutes in the reaction system is defined as 1 unit.

LDH activity (U/g_{prot}) = (ΔA_450 - b) ÷ a × f ÷ C_{pr} × 1000*

[Note]

ΔA_450: $OD_{sample} - OD_{control}$

f: Dilution factor of sample before test

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

1000*: 1 L = 1000 mL

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 Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	14.50	250.00	662.00
%CV	2.3	1.6	1.5

Inter-assay Precision

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

Inter-assay Precision Results

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	14.50	250.00	662.00
%CV	2.2	2.4	2.6

Recovery

Three samples of high, medium, and low concentrations were tested with 6 parallel measurements for each concentration to obtain an average recovery rate of 98%.

Recovery Results

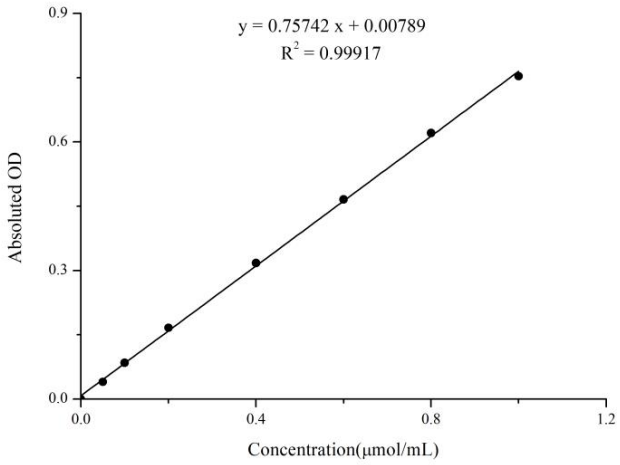
Standard	Expected Conc. (µmol/mL)	Observed Conc. (µmol/mL)	Recovery Rate (%)
Standard 1	0.08	0.1	98
Standard 2	0.24	0.2	99
Standard 3	0.76	0.7	97

Sensitivity

The analytical sensitivity of the assay is 6 U/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 values of the standard curve may vary according to actual assay performance conditions (e.g., operator, pipetting technique, or temperature effects), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (µmol/mL)	Average OD	Absolute OD
0	0.091	0.000
0.05	0.131	0.04

Concentration (μmol/mL)	Average OD	Absolute OD
0.1	0.175	0.084
0.2	0.257	0.166
0.4	0.408	0.317
0.6	0.557	0.466
0.8	0.712	0.621
1	0.844	0.753

11. Appendix II Example Analysis

Example analysis:

For human serum, dilute human serum with PBS (0.01 M, pH 7.4) by 10-fold, take 0.02 mL of diluted sample and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.75742x + 0.00789

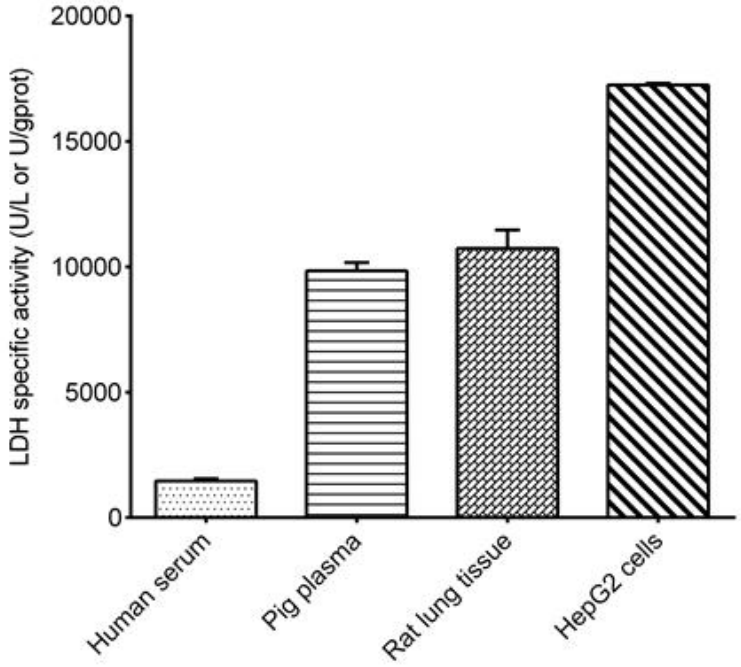
Average OD value of the sample: 0.232

Average OD value of the control: 0.115

Result:

LDH activity (U/L) = (0.232 - 0.115 - 0.00789) ÷ 0.75742 × 10 × 1000 = 1440.548 U/L

Detecting human serum (diluted 10-fold), porcine plasma (diluted 50-fold), 10% rat lung tissue homogenate (protein concentration: 6.51 g_{prot}/L, diluted 500-fold), and HepG2 cells (protein concentration: 3.41 g_{prot}/L, diluted 20-fold) according to the protocol yields the following results:



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 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 pretest 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.