TECHNICAL MANUAL

Lactate dehydrogenase (LDH) Activity Assay Kit (WST-8)

- **SKU CODE:** MAES0195
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Standard curve preparation
- Add standards and samples
- Incubate at 37°C
- Add stop solution
- Measure OD values at 450 nm

2. Intended use

This kit can be used to measure lactate dehydrogenase (LDH) activity in tissues, serum (plasma), hydrothorax, cells, and cell culture supernatant samples.

3. Detection principle

Lactate dehydrogenase catalyzes the reaction of lactic acid with NAD+ to produce pyruvic acid and NADH. NADH, under the action of PMS, transfers electrons to WST-8 to produce a yellow product, which has a characteristic absorption peak at 450 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Lysis Solution	60 mL × 1 vial	60 mL × 2 vials	-20°C, 12 months
Reagent 2	Substrate	1.5 mL × 1 vial	1.5 mL × 2 vials	-20°C, 12 months
Reagent 3	Chromogenic Agent	1.5 mL × 1 vial	1.5 mL × 2 vials	-20°C, 12 months, protect from light
Reagent 4	Coenzyme	Powder × 1 vial	Powder × 1 vial	-20°C, 12 months
Reagent 5	Stop Solution	3 mL × 1 vial	6 mL × 1 vial	-20°C, 12 months
Reagent 6	NADH Standard	Powder × 1 vial	Powder × 1 vial	-20°C, 12 months
	Microplate	48 wells	96 wells	No requirement

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Micropipettor, Vortex mixer, Incubator, Centrifuge, Microplate reader (450 nm)

Reagents:

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

6. Reagent preparation

1. Preheat stop solution at 37°C for 20 min in advance and use only after it is completely clarified. Equilibrate other reagents to room temperature before use.
2. **Preparation of coenzyme working solution:** Dissolve one vial of coenzyme with 260 µL of double distilled water, mix well to dissolve. The coenzyme working solution should be prepared fresh. Store at -20°C for 7 days protected from light.
3. **Preparation of reaction working solution:** For each well, prepare 50 µL of reaction working solution (mix well 24 µL of substrate, 24 µL of chromogenic agent and 2 µL of coenzyme working solution). The reaction working solution should be prepared fresh and stored protected from light.
4. **Preparation of 5 mmol/L standard solution:** Dissolve one vial of NADH standard with 2 mL of double distilled water, mix well to dissolve. The 5 mmol/L standard solution should be prepared fresh. Store at -20°C for 7 days protected from light.
5. **The preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 5 mmol/L NADH standard with lysis solution to a serial concentration. The recommended dilution gradient is as follows: 400, 300, 250, 200, 150, 100, 50, 0 µmol/L.

7. Sample preparation

Sample preparation:

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

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 lysis solution with a dounce homogenizer at 4°C.

4. Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

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

Cell (adherent or suspension) samples:

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

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

3. Lyse 1×10^6 cells with 400 µL lysis solution. Place on ice and mix well every 5 min, lyse for 10 min.

4. Centrifuge at 15000×g for 10 min. Collect supernatant and keep it on ice for detection.

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

Cell culture supernatant: Collect fresh cell culture supernatant and centrifuge at 10000×g for 10 min at 4°C. Take the supernatant and preserve it on ice for detection.

Dilution of sample:

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

Sample type - Dilution factor

Human serum: 10-20

Dog serum: 10-20

Mouse serum: 50-100

Cynomolgus monkey serum: 10-20

10% Rat spleen tissue homogenate: 150-250

10% Rat liver tissue homogenate: 250-350

10% Rat kidney tissue homogenate: 250-350

10% Rat lung tissue homogenate: 250-350

Note: The diluent is lysis solution. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

There should be no bubbles in the wells of the microplate when measuring the OD value.

9. Operating steps

1. Standard well: Take 50 µL of standard solution with different concentrations into the corresponding wells. Sample well: Take 50 µL of sample into the corresponding wells.
2. Add 50 µL of reaction working solution to each well.
3. Incubate at 37°C for 10 min.
4. Add 50 µL of stop solution to each well.
5. Mix thoroughly for 5 s with microplate reader. Measure the OD values of each well with microplate reader at 450 nm.

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 by using absolute OD value of standard and correspondent 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), cell culture supernatant and other liquid sample:

Unit definition: the enzyme amount of 1 µmol of NADH generated by 1 L of liquid sample per minute at 37°C is defined as 1 unit.

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

2. Tissue and cells sample:

Unit definition: the enzyme amount of 1 µmol of NADH generated by 1 g tissue protein or cell protein per minute at 37°C is defined as 1 unit.

LDH activity (U/gprot) = (ΔA_450 - b) ÷ a ÷ T × f ÷ C_{pr}

[Note]

ΔA_450: OD_{Sample} - OD_{Blank}.

f: Dilution factor of sample before test.

T: Reaction time (10 min).

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

11. Performance Characteristics

Parameter:

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.60	18.50	26.00
%CV	2.6	2.3	2.0

Inter-assay Precision

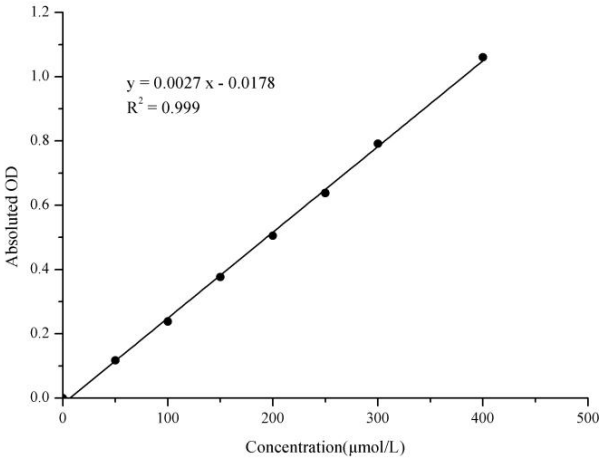
Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.60	18.50	26.00
%CV	2.4	2.5	2.0

Sensitivity

The analytical sensitivity of the assay is 0.11 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 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 as below for reference only:



12. Example Analysis

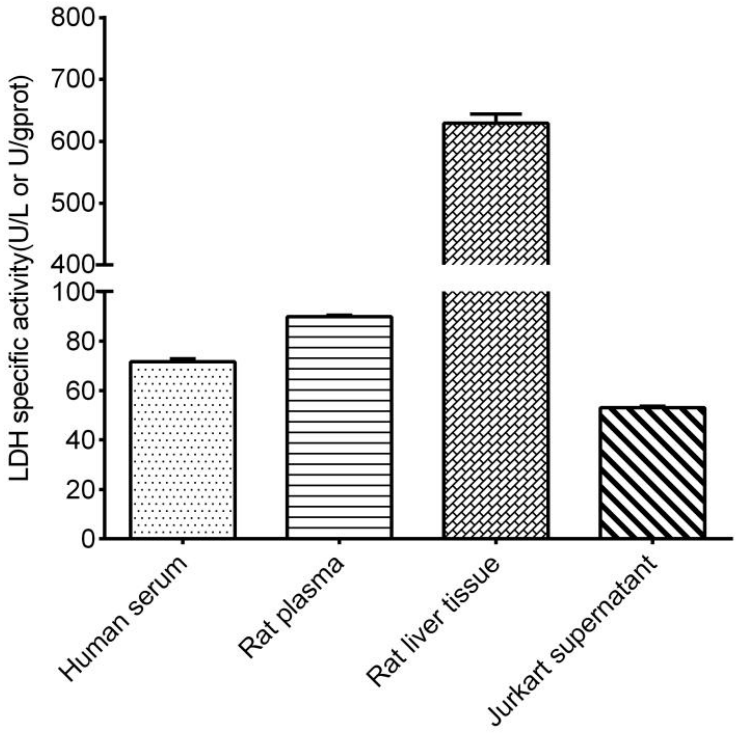
Example analysis:

Take 50 μL of human serum diluted 10 times, and carry out the assay according to the operation table. The results are as follows:

Standard curve: y = 0.0027x - 0.0178, the average OD value of the sample is 0.229, the average OD value of the blank is 0.054, and the calculation result is:

LDH activity (U/L) = (0.229 - 0.054 + 0.0178) ÷ 0.0027 ÷ 10 × 10 = 71.4 U/L

Detect human serum (dilute 10 times), rat plasma (dilute 10 times), 10% rat kidney tissue homogenate (the concentration of protein is 14.62 gprot/L dilute 250 times) and Jurkat supernatant (dilute 3 times) according to the protocol, the result is as follows:



13. 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.