Technical Manual

Lactate dehydrogenase (LDH) Colorimetric Assay Kit (WST-8 method)

- Catalogue Code: MAES0195
- Size: 96T
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.11-39.9 U/L

Sensitivity:

0.11 U/L

Storage:

-20°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Lactate dehydrogenase (LDH) is an oxidoreductase. LDH catalyzes the conversion of lactate to pyruvic acid and back, as it converts NAD^+ to NADH and back. LDH is composed of four subunits (tetramer). The two most common subunits are the LDH-M and LDH-H protein. LDH is released into the blood by cells after tissue damage or erythrocyte hemolysis. Extracellular LDH activity is used to detect cell damage or cell death.

3. Intended Use

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

4. Detection Principle

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

5. Kit Components & Storage

Item	Specification	Storage
Lysis Solution	60 mL × 2 vials	-20°C, 6 months
Substrate	1.5 mL × 2 vials	-20°C, 6 months
Chromogenic Agent	1.5 mL × 2 vials	-20°C, 6 months, avoid direct sunlight
Coenzyme	Lyophilized × 1 vial	-20°C, 6 months
Stop Solution	6 mL × 1 vial	-20°C, 6 months
NADH Standard	Lyophilized × 1 vial	-20°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Vortex mixer
- Water bath
- Microplate Reader (450 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

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

7. Reagent Preparation:

1. Bring all reagents to room temperature before use. Preheat stop solution at 37°C for 20 min in advance and can be used only after it is completely clarified.

2. Preparation of **coenzyme working solution:** Dissolve a vial of powder with 0.26 mL double distilled water. Prepare fresh solution before use and can be stored at -20℃ for 1 week.

3. Preparation of **reaction working solution:** Mix substrate, chromogenic agent, coenzyme working solution at a ratio of 12:12:1. Prepare the needed fresh solution before use and store it with shading light.

4. Preparation of **standard solution (5 mmol/L):** Dissolve a vial of powder with 2 mL double distilled water and mix fully. Prepare fresh solution before use and can be stored at -20°C for 1 week.

8. Sample Preparation

1. Serum sample:

Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:

Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g 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) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Hydrothorax:

Collect the fresh hydrothorax to the tubes with anticoagulant (heparin is recommended as an anticoagulant) and mix fully. Centrifuge the sample at 10000 g for 10 min, then take the supernatant and preserve on ice before detection.

4. Cell sample:

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (4×10^6): lysis solution (μL) =1: 400. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve on ice before detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

5. Tissue sample:

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of lysis solution (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 and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.11-39.9 U/L).

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.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 450 nm

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.

10. Operation Steps

The preparation of standard curve

Dilute 5 mmol/L NADH standard with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 400, 300, 250, 200, 150, 100, 50, 0 μmol/L.

The measurement of samples

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 fully for 5 s with microplate reader. Measure the OD values of each well with microplate reader at 450 nm.

Operation Table

	Standard well	Sample well
Sample (μL)		50
Standard solution with different concentrations (μL)	50	
Reaction working solution (μL)	50	50
Incubate at 37°C for 10 min		
Stop Solution (μL)	50	50
Mix fully for 5 s with microplate reader. Measure the OD values of each well with microplate reader at 450 nm.		

11. Calculations

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) 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

$$\text{LDH activity (U/L)} = \frac{\Delta A_{450} - b}{a} \div T \times f$$

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

$$\text{LDH activity (U/gprot)} = \frac{\Delta A_{450} - b}{a} \div T \times f \div C_{pr}$$

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_{450}: $OD_{Sample} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0)
T: Reaction time (10 min)
f: Dilution factor of sample before test.
C_{pr}: Concentration of protein in sample, gprot/L.

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12. Performance Characteristics

Detection Range	0.11-39.9 U/L
Sensitivity	0.11 U/L
Average inter-assay CV (%)	2.3
Average intra-assay CV (%)	2.3

Analysis

Take 50 µL of human serum diluted for 10 times, and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.0027 x – 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:

$$\text{LDH activity (U/L)} = (0.229 - 0.054 + 0.0178) \div 0.0027 \div 10 \times 10$$

$$= 71.4 \text{ U/L}$$

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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Notes:

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Notes:

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