TECHNICAL MANUAL

NADP-Malate Dehydrogenase (NADP-MDH) Activity Assay Kit

- **SKU CODE:** MAES0227
- **SIZE:** 48 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
- Add reaction working solution
- Add chromogenic agent
- Incubate and measure absorbance

2. Intended use

This kit can be used to measure NADP-malate dehydrogenase (NADP-MDH) activity in serum (plasma), animal tissue, and cell samples.

3. Detection principle

Malate dehydrogenase (MDH) is widely present in animals, plants, bacteria, and other organisms, and is one of the key enzymes in the tricarboxylic acid cycle, catalyzing the reversible conversion between malic acid and oxaloacetic acid. MDH plays an important role in various physiological activities of cells, including mitochondrial energy metabolism and reactive oxygen species metabolism in plants. MDH can be classified as NAD-dependent MDH or NADP-dependent MDH according to different coenzyme specificities. NADP-MDH usually exists in eukaryotic cells.

NADP-MDH catalyzes the conversion of malic acid and NADP+ to oxaloacetic acid and NADPH. NADPH causes WST-8 to turn orange in the presence of an electron coupling agent. The activity of NADP-MDH can be calculated by measuring the change in absorbance at 450 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extracting Solution	60 mL × 1 vial	60 mL × 2 vials	-20°C, 12 months
Reagent 2	Buffer Solution A	15 mL × 1 vial	30 mL × 1 vial	-20°C, 12 months

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 3	Substrate A	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, protect from light
Reagent 4	Substrate B	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, protect from light
Reagent 5	Chromogenic Agent	3 mL × 1 vial	6 mL × 1 vial	-20°C, 12 months, protect from light
Reagent 6	Standard	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, protect from light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

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

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate A stock solution::** Dissolve one vial of substrate A with 1 mL double-distilled water. Store aliquots at -20°C for up to 5 days, avoiding repeated freeze/thaw cycles.
3. **Preparation of substrate A working solution::** For each well, prepare 140 µL of substrate A working solution by mixing 9 µL of substrate A stock solution with 131 µL of buffer solution A. The substrate A working solution should be prepared fresh and used within 1 day.
4. **Preparation of substrate B working solution::** Dissolve one vial of substrate B with 5 mL double-distilled water. Store at -20°C for up to 5 days protected from light.
5. **Preparation of reaction working solution::** For each well, prepare 180 µL of reaction working solution by mixing 140 µL of substrate A working solution with 40 µL of substrate B working solution. The reaction working solution should be prepared fresh and used within 1 hour.

6. **Preparation of 500 µmol/L standard solution::** Dissolve one vial of standard powder with 1.6 mL extracting solution. Store at -20°C for up to 5 days protected from light, avoiding repeated freeze/thaw cycles.
7. **Preparation of standard curve::** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 500 µmol/L standard solution with extracting solution to create a serial concentration. The recommended dilution gradient is: 0, 100, 150, 200, 300, 400, 450, 500 µmol/L.

7. Standard curve preparation

Item	1	2	3	4	5	6	7	8
Concentration (µmol/L)	0	100	150	200	300	400	450	500
500 µmol/L Standard solution (µL)	0	40	60	80	120	160	180	200
Extracting solution (µL)	200	160	140	120	80	40	20	0

8. Sample preparation

Sample preparation:

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

Tissue sample:

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

2. Homogenize 20 mg tissue in 180 µL extracting solution with a Dounce homogenizer at 4°C.

3. Centrifuge at 10,000×g for 15 minutes to remove insoluble material. Collect supernatant and keep on ice for detection.

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

Cells:

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. Homogenize 1×10^6 cells in 200 µL extracting solution with an ultrasonic cell disruptor at 4°C.

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

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

9. Sample dilution

Sample type	Dilution factor
10% Rat kidney tissue homogenate	3-10
10% Rat heart tissue homogenate	1-5
10% Mouse liver tissue homogenate	1-10
10% Mouse lung tissue homogenate	3-10
10% Mouse kidney tissue homogenate	3-10
10% Epipremnum aureum tissue homogenate	1
Mouse serum	1
Rat serum	1
Human serum	1
Rat plasma	1
HT29 cell	1
Molt-4 cell	1

10. The key points of the assay

Avoid bubbles when adding reaction working solution.

11. Operating steps

1. Standard wells: Add 20 μL of standard solution with different concentrations to the corresponding wells. Sample wells: Add 20 μL of sample to the corresponding wells.
2. Add 180 μL of reaction working solution to each well.
3. Add 40 μL of chromogenic agent to each well.
4. Mix thoroughly with microplate reader for 3 seconds and incubate at room temperature protected from light for 2 minutes. Measure the OD value at 450 nm with a microplate reader, recorded as A1.
5. Incubate at 37°C for 10 minutes protected from light. Measure the OD value of sample wells and standard wells at 450 nm with a microplate reader, recorded as A2, ΔA = A2 - A1. (Note: There is no change in OD value of standard wells; plot the standard curve with the A2 OD values of standards).

12. 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 values of standards as the y-axis and corresponding concentrations as the x-axis. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

1. Serum (plasma) sample:

Definition: The amount of NADP-MDH in 1 L liquid sample per minute that hydrolyzes malic acid to produce 1 μmol NADPH at 37°C is defined as 1 unit.

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

2. Tissue and cell samples:

Definition: The amount of NADP-MDH in 1 g tissue or cell protein per minute that hydrolyzes malic acid to produce 1 μmol NADPH at 37°C is defined as 1 unit.

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

[Note]

ΔA_450: The change in OD values of sample wells (A_2-A_1).

T: The incubation reaction time, 10 minutes.

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

f: Dilution factor of sample before test.

13. 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 (U/L)	8.5	15.7	32.5
%CV	1.3	1.0	0.7

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	8.5	15.7	32.5
%CV	6.8	6.2	6.2

Recovery

Three samples of high, middle, and low concentrations were tested with each concentration analyzed 6 times in parallel to obtain an average recovery rate of 103%.

Recovery

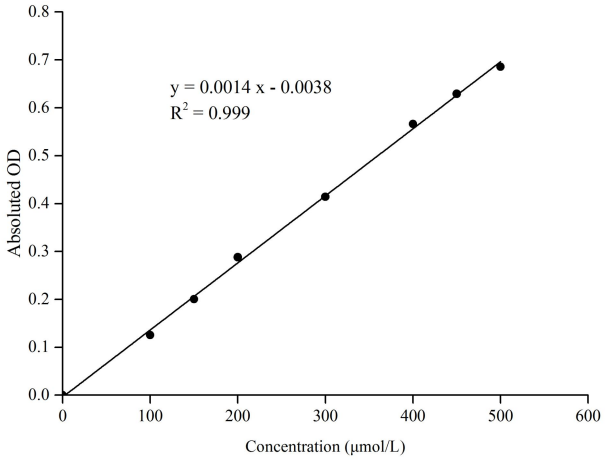
Parameters	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/L)	124	253	421
Observed Conc. (µmol/L)	130.2	255.5	433.6
Recovery rate (%)	105	101	103

Sensitivity

The analytical sensitivity of the assay is 0.63 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 below for reference only:



Standard curve

Concentration (µmol/L)	OD value 1	OD value 2	Average OD	Absolute OD
0	0.060	0.062	0.061	0.000
100	0.189	0.184	0.187	0.126
150	0.263	0.26	0.262	0.201

Concentration (μmol/L)	OD value 1	OD value 2	Average OD	Absolute OD
200	0.35	0.348	0.349	0.288
300	0.482	0.468	0.475	0.414
400	0.64	0.614	0.627	0.566
450	0.689	0.691	0.690	0.629
500	0.757	0.736	0.747	0.686

14. Appendix II Example Analysis

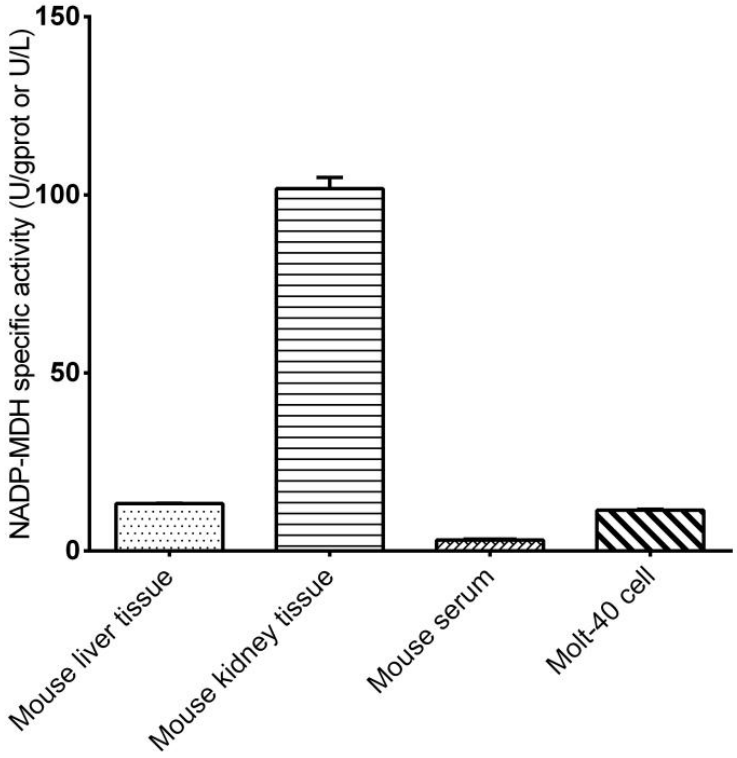
Example analysis:

For 10% mouse liver tissue homogenate, diluted 7-fold, and assayed according to the operation steps. The results are as follows:

Standard curve: y = 0.0014x - 0.0038, the OD value of sample A_1 is 0.131, the OD value of sample A_2 is 0.389, the concentration of protein in sample is 9.833 gprot/L, and the calculation result is:

NADP-MDH activity (U/gprot) = (0.389 – 0.131 + 0.0038) ÷ 0.0014 ÷ 10 × 7 ÷ 9.8 = 13.32 U/gprot

Detection of 10% mouse liver tissue homogenate (protein concentration: 9.83 gprot/L), 10% mouse kidney tissue homogenate (protein concentration: 4.20 gprot/L), mouse serum, and Molt-4 cells (protein concentration: 0.557 gprot/L) according to the protocol yields the following results:



15. 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.