TECHNICAL MANUAL

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

- **SKU CODE:** MAES0226
- **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
- Measure absorbance at 450 nm
- Incubate and measure final absorbance

2. Intended use

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

3. Detection principle

Malate dehydrogenase exists widely in animals, plants, bacteria, and other organisms, and is one of the key enzymes in the tricarboxylic acid cycle. It catalyzes the reversible conversion between malic acid and oxaloacetic acid. MDH can be divided into NAD-dependent MDH and NADP-dependent MDH according to different coenzyme specificities. NAD-MDH usually exists in the cytoplasm and mitochondria of bacteria and eukaryotic cells.

NAD-MDH catalyzes the conversion of substrate malic acid and NAD to oxaloacetic acid and NADH. NADH makes WST-8 orange under the action of electron coupling agent. The activity of NAD-MDH can be calculated by measuring the change of absorbance value 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	10 mL × 1 vial	20 mL × 1 vial	-20°C, 12 months

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 3	Buffer Solution B	5 mL × 1 vial	10 mL × 1 vial	-20°C, 12 months
Reagent 4	Substrate	Powder ×1 vial	Powder ×1 vial	-20°C, 12 months, shading light
Reagent 5	Chromogenic Agent	3 mL × 1 vial	6 mL × 1 vial	-20°C, 12 months, shading light
Reagent 6	Standard	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Incubator, centrifuge, 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 working solution:** Dissolve one vial of substrate with 5 mL double distilled water. Store aliquoted at -20°C for 5 days, and avoid repeated freeze/thaw cycles.
3. **Preparation of reaction working solution:** For each well, prepare 160 µL of reaction working solution (mix well 100 µL of buffer solution A, 40 µL of buffer solution B and 20 µL of substrate working solution). The reaction working solution should be prepared immediately before use and used within 1 hour.
4. **Preparation of 500 µmol/L standard solution:** Dissolve one vial of standard powder with 1.6 mL extracting solution. Store aliquoted at -20°C for 5 days, and avoid repeated freeze/thaw cycles.
5. **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 a serial concentration. The recommended dilution gradient is as follows: 0, 100, 150, 200, 300, 350, 400, 500 µmol/L. Reference is as follows:

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. 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 it on ice for detection.

4. Meanwhile, determine the protein concentration of 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 it 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):

10% Rat kidney tissue homogenate: 1

10% Rat spleen tissue homogenate: 1

10% Rat liver tissue homogenate: 1

10% Rat heart tissue homogenate: 1

10% Mouse liver tissue homogenate: 1

10% Mouse lung tissue homogenate: 1

Mouse serum: 1

Rat serum: 1

Mouse plasma: 1

Rat plasma: 1

Human serum: 1

Dog serum: 1

HT29 cell: 1

Molt-4 cell: 1

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

8. The key points of the assay

Avoid bubbles when adding reaction working solution.

9. Operating steps

1. Standard well: Add 20 µL of standard solution with different concentrations to the corresponding wells. Sample well: Add 20 µL of sample to the corresponding wells.
2. Add 160 µL of reaction working solution to each well.
3. Add 40 µL of chromogenic agent to each well.
4. Mix fully with microplate reader for 3 s and stand at room temperature protected from light for 2 min. Measure the OD value of sample well at 450 nm with microplate reader, recorded as A1.
5. Incubate at 37°C for 10 min protected from light. Measure the OD value of sample well and standard well at 450 nm with microplate reader, recorded as A2, ΔA = A2 - A1. (Note: There is no change in OD value of standard well, plot the standard curve with the OD value of A2(standard)).

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) sample:

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

NAD-MDH activity (U/L) = (ΔA450 - b) ÷ a ÷ T × f

2. Tissue sample and cell sample:

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

NAD-MDH activity (U/gprot) = (ΔA450 - b) ÷ a ÷ T ÷ C_{pr} × f

[Note]

ΔA450: The change OD values of sample well (A_2-A_1).

T: The time of incubation reaction, 10 min.

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

f: Dilution factor of sample before test.

11. 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)

Inter-assay Precision

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

Recovery

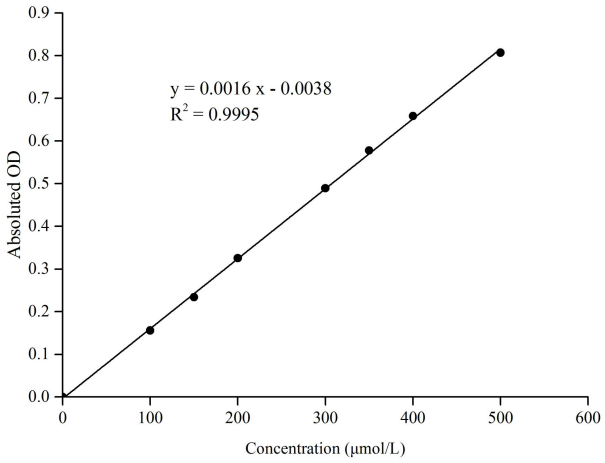
Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 96%.

Sensitivity

The analytical sensitivity of the assay is 1.06 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), so the standard curve and data are provided as below for reference only:



12. Appendix II Example Analysis

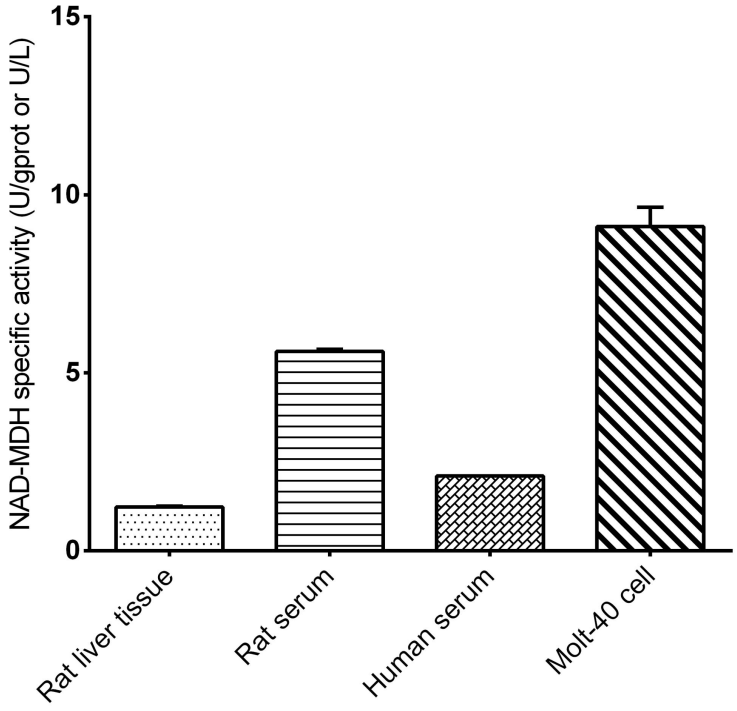
Example analysis:

For rat liver tissue, take 20 μL of 10% rat liver tissue homogenate, and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0016 x - 0.0038, the OD value of the sample A_1 is 0.52, the OD value of the sample A_2 is 0.701, the concentration of protein in sample is 8.63 gprot/L, and the calculation result is:

NAD-MDH activity (U/gprot) = (0.701 – 0.52 + 0.0038) ÷ 0.0016 ÷ 10 ÷ 8.63 = 1.33 U/gprot

Detect 10% rat liver tissue homogenate (the concentration of protein is 8.63 gprot/L), rat serum, human serum and Molt-40 cell (the concentration of protein is 0.619 gprot/L) 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. Please 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.