Technical Manual

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

- Catalogue Code: MAES0226
- Size: 96T
- Research Use Only

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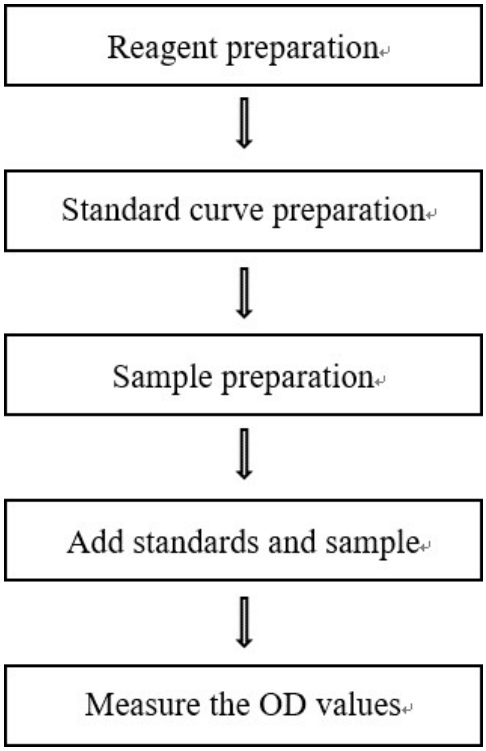
Assay summary



Intended use

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

Detection principle

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

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
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	No requirement
	Microplate	96 wells		
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of substrate working solution：

Dissolve a vial of substrate with 5 mL double distilled water. Aliquoted storage at -20°C for 5 days, and avoid repeated freeze/thaw cycles is advised.

③ The 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 20μL of substrate workingsolution). The reaction working solution should be prepared on spot, and use up within 1 h.

④ The preparation of 500 μmol/L standard solution：

Dissolve a vial of standard power with 1.6 mL extracting solution. Aliquotedstorage at -20°C for 5 days, and avoid repeated freeze/thaw cycles is advised.

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutionsafter 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:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**100**	**150**	**200**	**300**	**350**	**400**	**500**
500 μmol/L Standard solution (μL)	0	20	30	40	60	70	80	100
Extracting solution (μL)	100	80	70	60	40	30	20	0

Sample preparation

① **Sample preparation**

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

Tissue sample:

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

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

③ Centrifuge at 10000×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.

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 a ultrasonic cell disruptor 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.

② Dilution of sample

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

Sample type	Dilution factor
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 do pretest to confirm the dilution factor.

The key points of the assay

Avoid bubbles when adding reaction working solution.

Operating steps

①Standard 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 A_1.

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 A_2, $\triangle A = A_2 - A_1$. (Note: There is no change in OD value of standard well, plot the standard curve with the OD value of A_2(standard)).

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 absoluted OD value.
3. Plot the standard curve by using absoluted 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 hydrolyze the malic acid to produce 1 μmol NADH at 37°C is defined as 1 unit.

$$\text{NAD-MDH activity (U/L)} = (\Delta A450 - b) \div a \div T \times f$$

2. **Tissue sample and cells sample:**

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

$$\text{NAD-MDH activity (U/gprot)} = (\Delta A450 - b) \div a \div T \div Cpr \times f$$

[Note]

ΔA_{450}: The change OD values of sample well (A_2-A_1).

T: The time of incubation reaction, 10 min.

Cpr: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determineprecision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	5.6	28.4	33.5
%CV	3.6	2.9	2.5

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (UL)	5.6	28.4	33.5
%CV	8.2	8.3	8.7

Recovery

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

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	125	264	375
Observed Conc. (μmol/L)	120	248.2	367.5
Recovery rate (%)	96	94	98

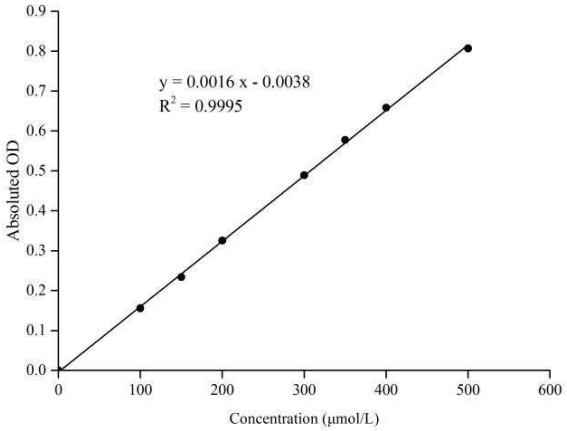
Sensitivity

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.

2. 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：

Concentration (μmol/L)	0	100	150	200	300	350	400	500
OD value	0.059	0.215	0.297	0.390	0.554	0.647	0.714	0.856
	0.059	0.215	0.289	0.379	0.543	0.627	0.721	0.876
Average OD	0.059	0.215	0.293	0.385	0.549	0.637	0.718	0.866
Absoluted OD	0.000	0.156	0.234	0.326	0.490	0.578	0.659	0.807



Appendix II Example Analysis

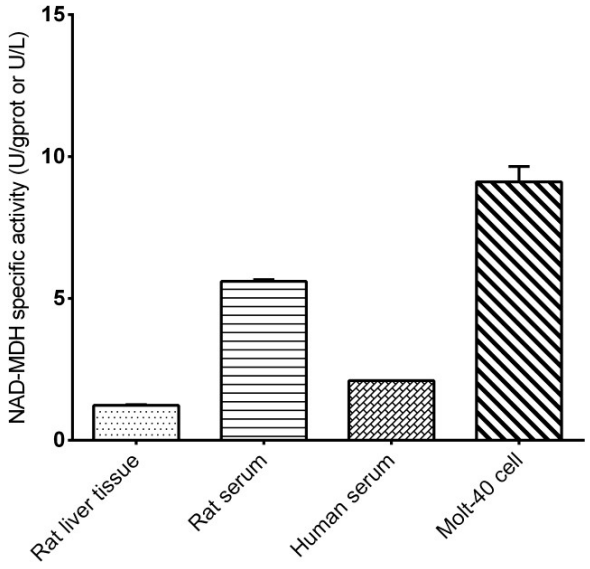
Example analysis：

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：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Assay Genie will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.

Notes

Notes

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.

Contact Details

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