（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K562-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（440-460 nm）

Detection range：0.63–50.0 U/L

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

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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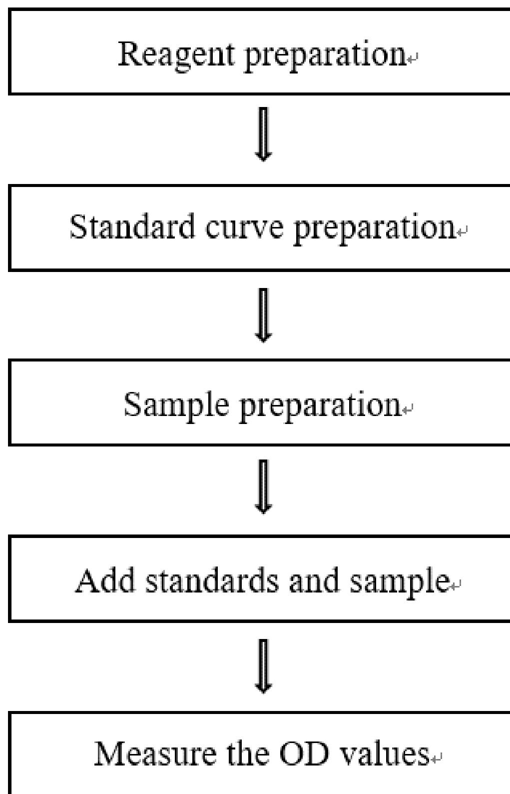
Assay summary



Intended use

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

Detection principle

Malate dehydrogenase (MDH) 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 plays an important role in various physiological activities of cells, including mitochondrial energy metabolism and reactive oxygen species metabolism in plants. MDH can be divided into NAD-dependent MDH and NADP-dependent MDH according to different coenzyme specificity. NADP-MDH usually exists in the eukaryotic cells.

NADP-MDH catalyzes the conversion of malic acid and $NADP^+$ to oxaloacetic acid and NADPH. NADPH makes WST-8 orange under the action of electron coupling agent. The activity of NADP-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 ℃, 12 months
Reagent 2	Buffer Solution A	15 mL ×1 vial	30 mL ×1 vial	-20 ℃, 12 months
Reagent 3	Substrate A	Powder ×1 vial	Powder ×2 vials	-20 ℃, 12 months shading light
Reagent 4	Substrate B	Powder ×1 vial	Powder ×2 vials	-20 ℃, 12 months shading light
Reagent 5	Chromogenic Agent	3 mL ×1 vial	6 mL ×1 vial	-20 ℃, 12 months shading light
Reagent 6	Standard	Powder ×1 vial	Powder ×2 vials	-20 ℃, 12 months shading light
	Microplate	96 wells		No requirement
	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, Microplate reader (440-460 nm, optimum wavelength: 450 nm)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate A stock solution：

Dissolve a vial of substrate A with 1 mL double distilled water. Aliquoted storage at -20 ℃ for 5 days, and avoid repeated freeze/thaw cycles is advised.

③ The preparation of substrate A working solution：

For each well, prepare 140 μL of substrate A working solution (mix well 9 μL

of substrate A stock solution and 131 μL of buffer solution A). The substrate A working solution should be prepared on spot, and use up within 1 day.

④ The preparation of substrate B working solution:

Dissolve a vial of substrate B with 5 mL double distilled water. Store at -20 ℃ for 5 days protected from light.

⑤ The preparation of reaction working solution:

For each well, prepare 180 μL of reaction working solution (mix well 140 μL of substrate A working solution and 40 μL of substrate B working solution). The reaction working solution should be prepared on spot, and use up within 1 h.

⑥ The preparation of 500 μmol/L standard solution::

Dissolve a vial of standard powder with 1.6 mL extracting Solution. Store at -20 ℃ for 5 days protected from light, and avoid repeated freeze/thaw cycles is advised.

⑦ The 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, 400, 450, 500 μmol/L. Reference is as follows:

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

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80 ℃ 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 ℃.

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 (E-BC-K318-M).

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 ℃.

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 (E-BC-K318-M).

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	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

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

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 180 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 with shading 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 with shading 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 NADP-MDH in 1 L liquid sample per 1 minute that hydrolyze the malic acid to produce 1 μmol NADPH at 37°C is defined as 1 unit.

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

2. Tissue sample and cells sample:

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

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

[Note]

ΔA_{450}: 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.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

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.

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

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 103%.

	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

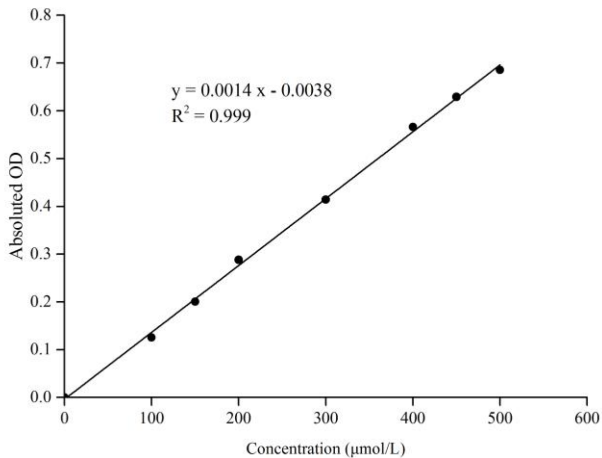
Sensitivity

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.

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**	**400**	**450**	**500**
OD value	0.060	0.189	0.263	0.35	0.482	0.64	0.689	0.757
	0.062	0.184	0.26	0.348	0.468	0.614	0.691	0.736
Average OD	0.061	0.187	0.262	0.349	0.475	0.627	0.690	0.747
Absoluted OD	0.000	0.126	0.201	0.288	0.414	0.566	0.629	0.686



Appendix Ⅱ Example Analysis

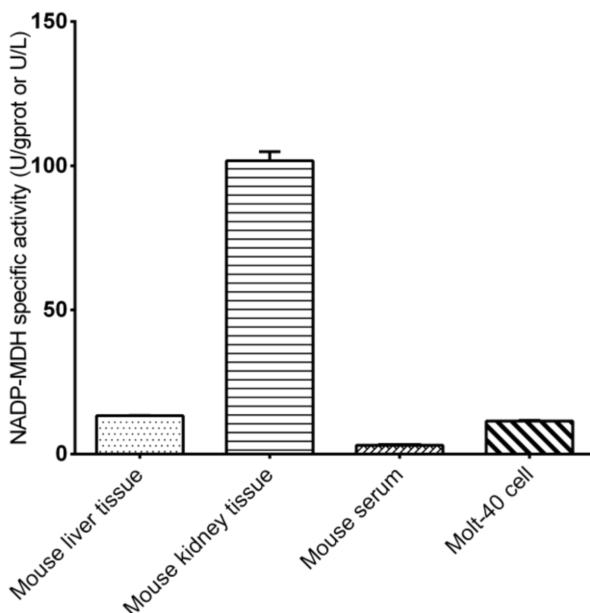
Example analysis:

For 10% mouse liver tissue homogenate, dilute for 7 times, and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.0014 x - 0.0038, the OD value of the sample A_1 is 0.131, the OD value of the 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

Detect 10% mouse liver tissue homogenate (the concentration of protein is 9.83 gprot/L), 10% mouse kidney tissue homogenate (the concentration of protein is 4.20 gprot/L), mouse serum and Molt-40cell (the concentration of protein is 0.557 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. Elabscience 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.