PRODUCT INFORMATION & MANUAL

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

NBP3-25888

For research use only.

Not for diagnostic or therapeutic procedures.

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

Catalog No: NBP3-25888

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.63 U/L

Detection range: 0.63–50.0 U/L

Average intra-assay CV (%): 1

Average inter-assay CV (%): 6.4

Average recovery rate (%): 103

General information

▲Intended use

This kit can 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	Specification	Storage
Reagent 1	Extracting Solution	60 mL × 2 vials	-20℃ , 12 months
Reagent 2	Buffer Solution A	30 mL × 1 vial	-20℃ , 12 months
Reagent 3	Substrate A	Powder × 2 vials	-20℃ , 12 months shading light
Reagent 4	Substrate B	Powder × 2 vials	-20℃ , 12 months shading light
Reagent 5	Chromogenic Agent	6 mL × 1 vial	-20℃ , 12 months shading light
Reagent 6	Standard	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.			

▲Materials prepared by users

Instruments

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

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

⚠ Precautions

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

▲ The key points of the assay

Avoid bubbles when adding reaction working solution.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 3 stock solution:

   Dissolve a vial of reagent 3 with 1 mL double distilled water. The prepared solution can be stored separately at -20°C for 5 days, avoid repeated freezing and thawing.

   Preparation of reagent 3 working solution:

   Mix the reagent 3 stock solution and reagent 2 at the ratio of 1:15 fully. Prepare the fresh needed amount before use and the prepared solution should be used within 1 day.
3. Preparation of reagent 4 working solution:

   Dissolve a vial of reagent 4 with 5 mL double distilled water. The prepared solution can be stored at -20°C for 5 days with shading light.
4. Preparation of reaction working solution:

   Mix the reagent 3 working solution and reagent 4 working solution at the ratio of 7:2 fully and preserve it on ice for detection. Prepare the fresh needed amount before use and the prepared solution should be used within 1 h.
5. Preparation of 500 μmol/L standard solution:

   Dissolve a vial of reagent 6 powder with 1.6 mL reagent 1. The prepared solution can be stored at -20°C for 5 days with shading light., avoid repeated freezing and thawing.

▲ Sample preparation

1. Serum and plasma samples:

Detect the sample directly.

2. Tissue sample:

Accurately weigh the tissue, add reagent 1 at a ratio of Weight (g): Volume (mL) =1:9 and homogenize the sample in ice water bath. Then centrifuge at 10000 g for 15 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant. If the supernatant is turbidity after centrifugation, repeated centrifuge until clear before use.

3. 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 (10^6): reagent 1 (μL) =1: 200. Sonicate or mechanical homogenate in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.63–50.0 U/L).

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 reagent 1.

Assay protocol

▲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.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 500 μmol/L standard solution with reagent 1 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:

Number	Standard concentrations (μmol/L)	500 μmol/L standard solution (μL)	Reagent 1 (μL)
A	0	0	200
B	100	40	160
C	150	60	140
D	200	80	120
E	300	120	80
F	400	160	40
G	450	180	20
H	500	200	0

2. The measurement of samples

(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 reagent 5 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, $\Delta A = A_2 - A_1$. (Note: There is no change in OD value of standard well, plot the standard curve with the OD value of $A_{2(Standard)}$).

▲Summary operation table

	Standard well	Sample well
Standard solution with different concentrations (μL)	20	
Sample (μL)		20
Reaction working solution (μL)	180	180
Regent 5 (μL)	40	40
Mix fully and stand at room temperature with shading light for 2 min. Measure the OD value of sample well, recorded as A_1.		
Incubate at 37℃ for 10 min with shading light. Measure the OD value of sample well and standard well at 450 nm, recorded as A_2, $\Delta A = A_2 - A_1$.		

▲Calculation

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. For tissue and cell :

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.

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

2. 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.

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

Note:

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

T: The time of incubation reaction, 10 min.

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

f: Dilution factor of sample before tested.

Appendix I Data

▲Example analysis

For 10% mouse liver tissue homogenate, dilute for 7 times, and carry the assay according to the operation table.

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 - 0.1312 + 0.0038) ÷ 0.0014 ÷ 10 × 7 ÷ 9.83 = 13.32 U/gprot