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

Catalog No：E-BC-K556-M

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

Measuring instrument：Microplate reader(340 nm)

Detection range：16.36-157.10 U/L

Elabscience® NAD-Malic Enzyme (NAD-ME) Activity Colorimetric 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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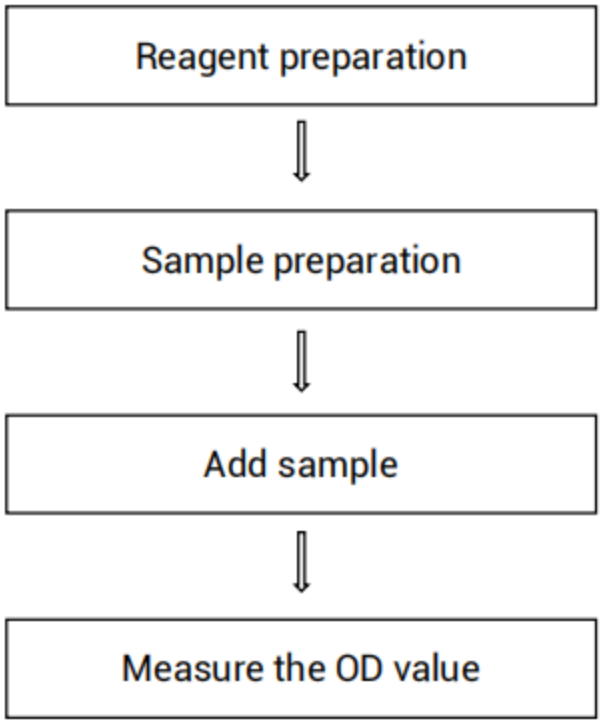
Assay summary



Intended use

This kit can be used to measure NAD-Malic enzyme (NAD-ME) activity in serum, animal tissue, plant tissue and cell samples.

Detection principle

Malic enzyme (ME) is widely present in both eukaryotes and prokaryotes and participates in various metabolic pathways. It mainly catalyzes the oxidation decarboxylation of malic acid to produce pyruvate and CO2, accompanied by the reduction of NAD (P) $^{+}$ to NAD (P) H. In mammalian cells, malic acid mainly includes ME1 in the cytoplasm that depends on NADP+, ME2 in the mitochondria that depends on NAD+, and ME3 in the mitochondria that depends on NADP+.

The detection principle of this kit: Malic enzyme catalyzes the substrate reaction to generate NADH. There is a characteristic absorption peak at 340 nm. By measuring the increase rate of absorbance at 340 nm, the activity of NAD-ME can be characterized.

Kit components & storage

Item	Component	Size1 (48 T)	Size1 (96 T)	Storage
Reagent 1	Extraction Solution	55 mL ×1vial	55 mL ×2 vials	-20℃, 12 months
Reagent 2	Buffer Solution	11 mL × 1 vial	22 mL × 1 vial	-20℃, 12 months, shading light
Reagent 3	Co-factor	Powder × 2 vials	Powder × 4 vials	-20℃, 12 months, shading light
	UV-Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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:

Microplate reader (340 nm), Incubator

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of co-factor working solution:
Dissolve one vial of co-factor with 0.6 mL of buffer solution, mix well to dissolve. Keep co-factor working solution on ice box protected from light for use. Aliquoted storage at -20°C for 7 days protected from light.

③ The preparation of reaction working solution:
For each well, prepare 190 μL of reaction working solution (mix well 170 μL of buffer solution and 20 μL of co-factor working solution). Store protected from light and used up within 8 h.

Sample preparation

① Sample preparation

Serum and plasma samples: detect directly (centrifuge at 10000 × g for 5 min and take the supernatant on ice for detection if there is turbidity)

Tissue samples:

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

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

③ Centrifuge at 10000 × g for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection and detect within 4 h.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Lyse 1×10^6 cells in 200 μL extraction solution and mix well every 5 min, lyse for 10 min.

③ Centrifuge at 10000 × g for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection and detect within 4 h.

④ 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% Mouse liver tissue homogenate	1
10% Mouse muscle tissue homogenate	1
10% Mouse spleen tissue homogenate	5-10
10% Mouse kidney tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Mouse lung tissue homogenate	4-10
10% Banana tissue homogenate	2-3
10% Apple tissue homogenate	1
1×10^6 Hela cells	1
1×10^6 A549 cells	1
1×10^6 Jurkat cells	1
Rat serum	1
Human serum	1
Mouse serum	1

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

The key points of the assay

① Avoid generating bubbles when adding the reaction working solution.

② Try to use fresh samples for the detection. The samples should be detected on the same day after processing.

③ The number of wells in each experiment should be controlled within 10.

④ If the value of the sample well is less than 0.005, appropriately extend the reaction time. The reaction time of the corresponding calculation formula needs to be modified.

Operating steps

① Sample well: Take 5 μL of sample into the sample wells.

② Add 190 μL of reaction working solution into the sample wells.

① Mix well with microplate reader for 5 s. Measure the OD values of each well at 340 nm with microplate reader, recorded as A_1.

② Incubate at 37°C for 3 min. Measure the OD values of each well at 340 nm with microplate reader, recorded as A_2.

Calculation

① **The serum samples**:

Definition: The amount of enzyme in 1 L serum per 1 min that produce 1 μmol NADH at 37°C is defined as 1 unit.

$$\text{NAD-ME activity (U/L)} = \Delta A_{sample} \div (\varepsilon \times d) \times \frac{V_{total}}{V_{sample}} \times 10\text{^}6* \div T \times f = \Delta A_{sample} \times 3483.38* \times f$$

② **The animal tissue or cell samples**:

Definition: The amount of enzyme in 1 g tissue or cell protein per 1 min that produce 1 μmol NADH at 37°C is defined as 1 unit.

$$\text{NAD-ME activity (U/gprot)} = \Delta A_{sample} \div (\varepsilon \times d) \times \frac{V_{total}}{V_{sample}} \times 10\text{^}6* \div T \div C_{pr} \times f = \Delta A_{sample} \times 3483.38* \div C_{pr} \times f$$

③ **The plant tissue samples**:

Definition: The amount of enzyme in 1 kg tissue per 1 min that produce 1 μmol NADH at 37°C is defined as 1 unit.

$$\text{NAD-ME activity (U/kg wet weight)} = \Delta A_{sample} \div (\varepsilon \times d) \times \frac{V_{total}}{V_{sample}} \times 10\text{^}6* \div T \div \frac{m}{v} \times f =$$

$$\Delta A_{sample} \times 31350.42* \times f$$

[Note]

ΔA_{sample}: The A_2 value of sample well - the A_1 value of sample well.

ε: The molar extinction coefficient of at 340 nm, 6.22 × 10^3 L·mol^{-1}·cm^{-1}.

d: Optical path, 0.6 cm.

V_{total}: The volume of reaction system, 0.195 mL.

V_{sample}: The volume of sample, 0.005 mL.

10^6*:1 mol = 1×10^6 μmol.

T: Reaction time, 3 min.

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

m: The weight of sample, kg.

v: The volume of extraction solution during homogenate, L.

f: Dilution factor of sample before tested.

3483.38*: Simplified value.

31350.42*: Simplified value.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse 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)	30.00	60.00	120.00
%CV	3.5	4.8	2.9

Inter-assay Precision

Three mouse serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	30.00	60.00	120.00
%CV	7.8	9.9	9.6

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 97.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	30.00	60.00	120.00
Observed Conc. (U/L)	30.0	59.4	112.8
Recovery rate (%)	100.0	99.0	94.0

Sensitivity

The analytical sensitivity of the assay is 16.36 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.

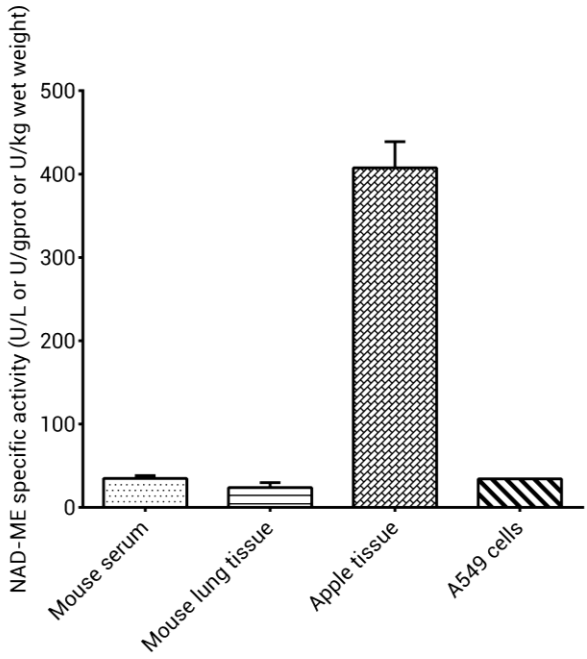
Appendix Π Example Analysis

Example analysis：

Take 5 μL of 10% mouse lung tissue homogenate (dilute for 4 times) and carry the assay according to the operation table. The results are as follows: A_1 of the sample well is 0.188, the A_2 of the sample well is 0.202, ΔA_{sample} = 0.202 - 0.188 = 0.014, the concentration of protein is 7.92 gprot/L and the calculation result is:

NAD-ME activity (U/gprot) = 0.014 × 3483.38 ÷ 7.92 × 4 = 24.64 U/gprot

Detect mouse serum,10% mouse lung tissue homogenate(the concentration of protein is 7.92 gprot/L, dilute for 4 times), 10% apple tissue homogenate, 1×10^6 A549 cells(the concentration of protein is 1.43 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.