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

Catalog No：E-BC-K555-M

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

Measuring instrument：Microplate reader(340 nm)

Detection range：2.68-338.96 U/L

Elabscience® NADP-Malic Enzyme (NADP-ME) Activity Colorimetric Assay Kit

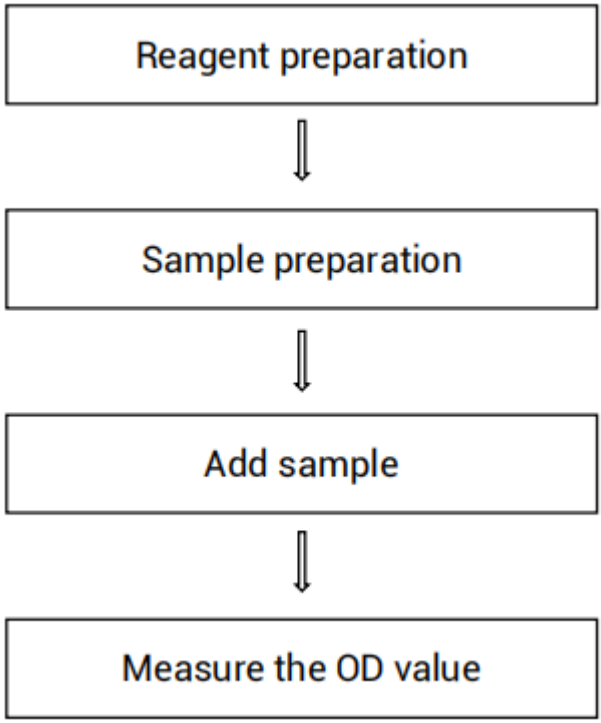
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 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.

Table of contents

Assay summary



Intended use

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

Detection principle

Malic enzyme (ME) is widely present in eukaryotes and prokaryotes and participates in multiple metabolic pathways. Its main metabolic substrate is malic acid, which mainly catalyzes the oxidation and decarboxylation of malic acid to pyruvate and CO_2, accompanied by the reduction of $NAD(P)^+$ to NAD(P)H. Malic acid is an important intermediate product in the tricarboxylic acid cycle. Under the catalysis of ME, malic acid is converted into pyruvate and NAD(P)H to regulate cellular energy, REDOX balance and the synthesis of biological macromolecules. In mammalian cells, malic enzymes mainly consist of NADP+ dependent ME1 in the cytoplasm, NAD+ dependent ME2 in the mitochondria, and NADP+ dependent ME3 in the mitochondria. Inhibiting ME1 can induce cellular senescence or apoptosis to suppress the growth of tumor cells.

The detection principle of this kit: The enzyme catalyzes the substrate reaction to generate NADPH, which has a characteristic absorption peak at 340 nm. The activity of NADP-ME can be determined by measuring the rate of increase in absorbance at 340 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	50 mL × 1 vial	50 mL × 2 vials	-20°C, 12 months shading light
Reagent 2	Buffer Solution	10 mL × 1 vial	20 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	Co-factor	Powder × 2 vials	Powder × 4 vials	-20°C, 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 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 it on ice and protected from light during use. The co-factor working solution can be aliquoted and stored at -20°C for 7 days protected from light.

③ The preparation of reaction working solution：
For each well, prepare 180 μL of reaction working solution (mix well 160 μL of buffer solution and 20 μL of co-factor working solution). Keep it on ice during use protected from light and used up within 8 h.

Sample preparation

① Sample preparation

Tissue sample:

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. The supernatant should be used within 4 h.

④ Meanwhile, determine the protein concentration of animal 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 with 200 μL extraction solution. Place on the ice box 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. The supernatant should be used within 4 h.

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

③ 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 kidney tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Mouse spleen tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse muscle tissue homogenate	1
10% Potato tissue homogenate	1
10% Corn tissue homogenate	1
10% Cauliflower tissue homogenate	1
1×10^6 RAW 264.7 cells	1
1×10^6 A549 cells	1
1×10^6 4T1 cells	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 bubbles when adding reaction working solution.

② The sample wells of each experiment should be less than 10.

Operating steps

① Sample well: add 20 μL of sample into sample wells.

② Add 180 μL of reaction working solution into sample wells.

③ Mix well for 5 s with microplate reader and measure the OD values of each well at 340 nm with microplate reader, as A_1.

④ Incubate at 37°C for 5 min and measure the OD value of each well at 340 nm with microplate reader, as A_2.

Calculation

The sample:

1. Animal tissue or cell samples:

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

$$\text{NADP-ME activity (U/gprot)} = \Delta A_{sample} \div (\varepsilon \times d) \times \frac{V_1}{V_2} \times 10\text{^}6 \div T \div C_{pr} \times f$$

$$= \Delta A_{sample} \times 535.91 \div C_{pr} \times f$$

2. Plant tissue samples:

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

$$\text{NADP-ME activity (U/kg wet weight)} = \Delta A_{sample} \div (\varepsilon \times d) \times \frac{V_1}{V_2} \times 10\text{^}6 \div T \div \frac{m}{v} \times f$$

$$= \Delta A_{sample} \times 4823.19 \times f$$

[Note]:

ΔA_{sample}: $\Delta A_{sample} = A_2 - A_1$.

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

d: Optical path, 0.6 cm.

V_1: The volume of reaction system, 0.2 mL.

V_2: The volume of sample added to the reaction system, 0.02 mL.

10^6 ：1 mol = 1×10^6 μmol

T: Reaction time, 5 min.

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

m: The weight of sample, g.

V: The volume of extraction solution in the preparation step of cell, mL.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse lung 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)	50.00	100.00	200.00
%CV	1.3	1.3	1.4

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	50.00	100.00	200.00
%CV	1.5	2.3	2.7

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 101%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	50.00	100.00	200.00
Observed Conc. (U/L)	51.5	100.0	198.0
Recovery rate (%)	103	100	99

Sensitivity

The analytical sensitivity of the assay is 2.68 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

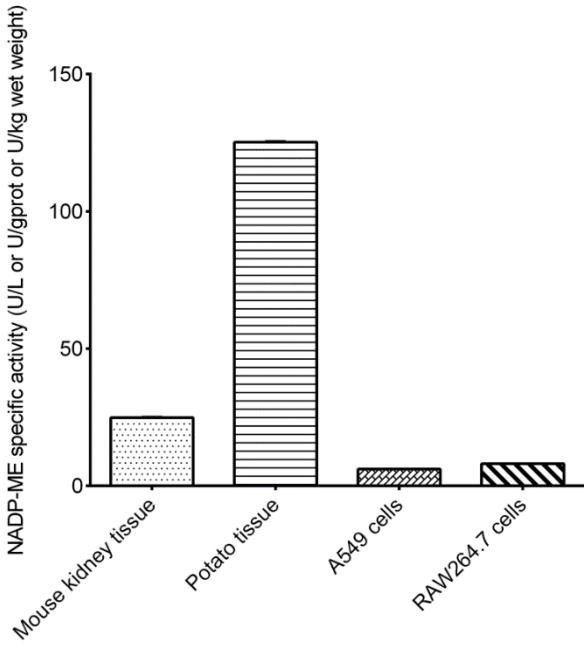
Example analysis：

Take 20 μL of 10% mouse kidney tissue homogenate and carry the assay according to the operation steps. The results are as follows:

The A_1 of the sample well is 0.427, the A_2 of the sample well is 0.945, ΔA_{sample} = 0.945 - 0.427 = 0.518, the concentration of protein is 11.14 gprot/L, and the calculation result is:

$$\text{NADP-ME activity (U/gprot)} = 0.518 \times 535.91 \div 11.14 \times 1 = 24.92 \text{ U/gprot}$$

Detect 10% mouse kidney tissue homogenate (the concentration of protein is 11.14 gprot/L), 10% potato tissue homogenate, 1×10^6 A549 cells (the concentration of protein is 1.23 gprot/L) and 1×10^6 RAW264.7 cells (the concentration of protein is 0.86 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.