

## **NAD Malic Enzyme(NAD-ME) Activity Assay Kit**

**Note:** Take two or three different samples for prediction before test.

**Detection equipment:**Spectrophotometer/Microplate reader

**Cat No:**NA0544

**Size:**100T/96S

### **Components:**

Extract solution: 110 mL×1, store at 4°C.

Reagent I:20 mL×1, store at 4°C.

Reagent II:Powder×1, store at4°C. Add 10mLof extract solution when the solution will be used.

Reagent III: Powder×1, store at 4°C. Add 1 mLof double distilled water when the solution will be used.

Reagent IV:Powder×1, store at -20°C. Add500μLof double distilled water when the solution will be used.  
other reagents store at 4°C.

Working solution: Add 1mLof Reagent III and 0.5mL of reagent IV to 7.5mL of Reagent II, prepare according the ratio when the solution will be used.

### **Description:**

MEwidely exists in microbe, culturing cells, animal and plantcytoplasm. Especially it has high activity in plant tissue. ME catalyzesthe reversible reaction of malic acidoxidative decarboxylation to produce pyruvic acid and CO<sub>2</sub>with the reduction of NAD(P)<sup>+</sup>, which is the crucial enzyme of malic acid metabolism. The activity of ME is related with biosynthesis and anti-oxidation. In recent years, the determination of plant ME activity become a hot spot in antioxidant research. According the coenzyme specificity and substrate specificity, ME is divided into NAD-ME(EC1.1.1.38) and NADP-ME(EC1.1.1.40).

NADP-ME catalyzesthe reduction of NADP<sup>+</sup> to NADPH,detect the increase rate of NADPH at 340nm.

### **Required but not provided**

Spectrophotometer/microplate reader, low temperature centrifuge, water bath, adjustable pipette, micro quartz cuvette/96 well flat-bottom plate(UV plate), mortar/homogenizer and distilled water.

### **Protocol:**

#### **I. Crude enzyme extraction:**

1. Germ or culturing cells:

Collect germ or/cells to centrifuge tube, discard the supernatant, add 1 mL of Extract solution to 5 million germ or cells, ultrasonic crush germ or cells (powder 20%, ultrasonic 3s, interval 10s, repeat 30 times). Centrifuge at 8000×g for 10min at 4°C, take the supernatant and keep it on ice for test.

2. Tissue:

Take 0.1 g of tissue, add 1mL of Extract solution, homogenate on ice bath. Centrifuge at 8000×g for 10min at 4°C, take the supernatant and keep it on ice for test.

Serum: Detect directly.

## II. Procedure

1. Preheat spectrophotometer/microplatereader for 30minutes, adjust wavelength to 340nm, set zero with distilled water.
2. Preheat Reagent I at 25°C(general species) or 37°C(mammals) water bath for 15 minutes.
3. Procedure test

Reagent(μL)	Test tube
Reagent I	200
Working solution	90
Sample	10

Add reagents to 1 mL micro quartz cuvette orderly, mix thoroughly. Record the initial absorbance A1 and absorbance A2 after 1min at 340 nm at 25°C(general species) or 37°C(mammals),  $\Delta A = A2 - A1$ .

### Note:

1. If  $A2 - A1 > 0.5$ , dilute enzyme solution with extract solution to make  $A2 - A1 < 0.5$  and increase detect sensitivity.
2. During experiment, place reagent III, reagent IV and sample on ice, avoid denaturation and lose activity. Reagent I is placed on 37°C or 25°C water bath.
3. The temperature of react solution must keep 37°C or 25°C, take a beaker, add 37°C or 25°C distilled water to the beaker, keep it at 37°C or 25°C water bath. Put the cuvette and reaction solution to the beaker during reaction process.
4. Two people do this experiment at the same time, one person colorimetric, the other person timing to ensure accuracy the results of the experiment.
5. If  $\Delta A < 0.01$ , prolong the reaction time to 5 minutes or 10 minutes.

## III. Calculation

### A. Microplate reader

1. Tissue

(1). Protein concentration

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the produce of 1nmol of NADH per minute every milligram of tissue protein.

$$\text{NAD-ME(U/mg prot)} = [\Delta A \times V_{RT} \div (\epsilon \times d) \times 10^9] \div (C_{Pr} \times V_{SA}) \div T = 965 \times \Delta A \div C_{Pr}$$

(2) Sample weight

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the produce of 1nmol NADH of per minute every gram of tissue.

$$\text{NAD-ME(U/g)} = [\Delta A \times V_{RT} \div (\epsilon \times d) \times 10^9] \div (V_{SA} \div V_{ST} \times W) \div T = 965 \times \Delta A \div W$$

2. Germ or cells

(1) Protein concentration

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the produce of 1nmol of NADH per minute every milligram of protein.

$$\text{NAD-ME (U/mg prot)} = [\Delta A \times V_{RT} \div (\epsilon \times d) \times 10^9] \div (\text{Cpr} \times V_{SA}) \div T = 965 \times \Delta A \div \text{Cpr}$$

(2) Germ or cells

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the produce of 1nmol of NADH per minute every 10 thousand germ or cells protein.

$$\text{NAD-ME (U/10}^4 \text{ cell)} = [\Delta A \times V_{RT} \div (\epsilon \times d) \times 10^9] \div (V_{SA} \div V_{RT} \times 500) \div T = 1.93 \times \Delta A$$

3. Serum

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the produce of 1nmol of NADH per minute every milliliter of serum.

$$\text{NAD-ME (U/mL)} = [\Delta A \times V_{RT} \div (\epsilon \times d) \times 10^9] \div V_{SA} \div T = 965 \times \Delta A$$

$V_{RT}$ : Total reaction volume,  $3 \times 10^{-4}$  L;

$\epsilon$ : Molar extinction coefficient,  $6.22 \times 10^3$  L/mol/cm;

$d$ : Cuvette light diameter (cm), 1 cm;

$V_{SA}$ : Sample volume, 0.01 mL;

$V_E$ : Extract solution volume, 1 mL;

$T$ : Reaction time (min), 5 minutes;

$\text{Cpr}$ : Sample protein concentration, mg/mL;

$W$ : Sample weight, g;

500: Cells or germ, 5 million.

### **B. 96 well plate:**

The optical diameter of 96 well plates in the above formula is changed to 0.6 cm for calculation.

### **Experimental instances:**

1. Take 0.1g of liver, add 1mL of extract solution, homogenate and grind. Take the supernatant and detect according to the measured steps. Calculate  $\Delta A = A_2 - A_1 = 0.893 - 0.6814 = 0.2116$ , calculate the enzyme activity according to sample weight:

$$\text{NAD-ME (U/g weight)} = 965 \times \Delta A \div W = 965 \times 0.2116 \div 0.1 = 2041.94 \text{ U/g weight.}$$

### **References:**

[1] Spampinato C P, Colombo S L, Andreo C S. Interaction of analogues of substrate with NADP-malic enzyme from maize leaves[J]. Photosynthesis research, 1994, 39(1): 67-73.

### **Related products:**

NA0843/NA0601	Coenzyme NAD(H) Content Assay Kit
NA0795/NA0554	NAD Kinase (NADK) Assay Kit
NA0818/NA0576	NADH oxidase (NOX) Activity Assay Kit