

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: BC1135

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 110 mL×1	2-8°C
Reagent I	Liquid 20 mL×1	2-8°C
Reagent II	Powder×1	2-8°C
Reagent III	Powder×1	2-8°C
Reagent IV	Powder×1	-20°C

Solution Preparation:

1. Reagent II: Add 10mL of Extract solution when the solution will be used.
2. Reagent III: Add 1 mL of distilled water when the solution will be used.
3. Reagent IV: Add 500µL of distilled water when the solution will be used.
4. Working solution: Add 1 mL of Reagent III and 0.5 mL of Reagent IV to 7.5 mL of Reagent II, prepare according to the ratio when the solution will be used.

Description:

ME widely exists in microbe, culturing cells, animal and plant cytoplasm. Especially it has high activity in plant tissue. ME catalyzes the reversible reaction of malic acid oxidative decarboxylation to produce pyruvic acid and CO₂ with the reduction of NAD(P)⁺, 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 has become a hot spot in antioxidant research. According to the coenzyme specificity and substrate specificity, ME is divided into NAD-ME (EC 1.1.1.38) and NADP-ME (EC 1.1.1.40).

NADP-ME catalyzes the reduction of NADP⁺ 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 1mLof 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 1nmolof NADHper 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 1nmolNADHof per minuteevery 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 1nmolof NADHper minute every milligram of protein.

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

(2) Germ or cells

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the produce of 1nmolof NADHper 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 1nmolof NADHper 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, 3×10^{-4} L;

ϵ : Molar extinction coefficient, 6.22×10^3 L/mol/cm;

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

V_{SA} : Sample volume, 0.01 mL;

V_E : Extract solution volume, 1mL;

T : Reaction time(min), 5minutes;

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:

BC0310/BC0315	CoenzymeINAD(H) Content Assay Kit
BC1030/BC1035	NAD Kinase (NADK) Assay Kit
BC0630/BC0635	NADH oxidase(NOX) Activity Assay Kit