NADP Malic Enzyme(NADP-ME) Activity Assay Kit

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

Detection equipment: Spectrophotometer

Cat No:NA0786

Size:50T/48S

Components:

Extract solution:70 mL×1, store at 4℃.

Reagent I:40 mL×1, store at 4℃.

Reagent II:Powder×1, store at4℃. Add 20mL of Extract solution when the solution will be used.

Reagent III: Powder×2, store at 4℃. Add 1 mLof distilled water when the solution will be used.

Reagent IV: Powder×2, store at -20℃.Add500μLof distilled water when the solution will be used.

Working solution: Add 2 mLof Reagent III and 1 mL of Reagent IV to 15 mL of Reagent II, prepare according the ratio when the solution will be used.

Description:

ME widely exist in microbe, culturing cells, animal and plant cytoplasm. Especially it has high activity in plant tissue. ME catalyzesthe reversible reaction of malic acidoxidative decarboxylation to produce pyruvic acid and $CO_2$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 become a hot spot in antioxidant research. According the coenzyme specificity and substrate specificity, MEis divided into NAD-ME(EC1.1.1.38) and NADP-ME(EC1.1.1.40).

NADP-ME catalyzesthe reduction of $NADP^+$ to NADPH,detect the increase rate of NADPH at 340nm.

Required but not provided

Spectrophotometer, low temperature centrifuge, water-bath, adjustable pipette, 1 mL quartz cuvette, 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 of germ or cells, ultrasonic crush germ or cells (powder 20%, ultrasonic 3s, interval 10s, repeat 30 times). Centrifuge at 8000×g for 10 minutes at 4℃, 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 10minutes at 4℃, take the supernatant and keep it on ice for test.

3. Serum: Detect directly.

II. Procedure

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

Reagent(μL)	Test tube
Reagent I	600
Working solution	270
Sample	30

Add reagents to 1 mL micro quartz cuvette orderly, mix thoroughly. Record the initial absorbance A1 and absorbance A2 after 1minute at 340 nm at 25℃(general species) or 37℃(mammals), ΔA=A2-A1.

Note:

1. If A2-A1>0.5, dilute enzyme solution with extract solution to make A2-A1<0.5and 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℃ or 25℃ water bath.
3. The temperature of react solution must keep 37℃ or 25℃, take a beaker, add 37℃ or 25℃ distilled water to the beaker, keep it at 37℃ or 25℃ 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 ΔA<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 enzymecatalyzes the produceof 1nmolof NADPHper minuteevery milligram of tissue protein.

NADP-ME(U/mg prot)=[ΔA×V_{RT}÷(ε×d)×10^9]÷(Cpr×V_{SA}) ÷T=4823×ΔA÷Cpr

(2) Sample weight

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the produce of1nmolof NADPHper minuteevery gram of tissue.

NADP-ME(U/g)=[ΔA×V_{RT}÷(ε×d)×10^9]÷(V_{SA}÷V_{ST}×W) ÷T =4823×ΔA÷W

2. Germ or cells

(1) Protein concentration

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the produce of1nmolof NADPHper minuteevery milligram of protein.

NADP-ME(U/mg prot)=[ΔA×V_{RT}÷(ε×d)×10^9]÷(Cpr×V_{SA}) ÷T=4823×ΔA÷Cpr

(2) Germ or cells

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the produce of1nmolof NADPHper minuteevery 10 thousand germ or cells.

NADP-ME(U/10^4 cell)=[ΔA×V_{RT}÷(ε×d)×10^9]÷(V_{SA}÷V_{RT}×500) ÷T=9.65×ΔA

(3) Serum

Unit definition: One unit of enzyme is defined as the amount of enzyme catalyzes the produce of1nmolof NADPHper minuteevery milliliter of serum.

NADP-ME(U/mL)=[ΔA×V_{RT}÷(ε×d)×10^9]÷V_{SA}÷T =4823×ΔA

V_{RT}: Total reaction volume, 9×10^{-4} L;

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

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

Vs_A:Sample volume, 0.03 mL;

$V_{E:}$ Extract solution volume, 1mL;

T: Reaction time(min), 1 minute;

Cpr:Sample protein concentration, mg/mL;

W:Sample weight, g;

500: Cells or germ, 5 million.

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. CalculateΔA=A2-A1=0.924-0.637=0.287, calculate the enzyme activity according to sample weight:

NADP-ME（U/g weight）=4823×ΔA÷W=4823×0.287÷0.1=13842.01 U/g weight.

2. Take 0.1g garlic, add 1mL of extract solution, homogenate and grind. Take the supernatant and detect according to the measured steps. CalculateΔA=A2-A1=0.171-0.135=0.036, calculate the enzyme activity according to sample weight:

NADP-ME（U/g weight）=4823×ΔA÷W=4823×0.036÷0.1=1736.28 U/g weight.

Recent Product citations

[1] BaohuaZhu,RuihaoZhang,NanaLv,et al. The Role of Malic Enzyme on Promoting Total Lipid and Fatty Acid Production in Phaeodactylumtricornutum. Frontier in Immunology. June 2018;(IF4.716)

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：

NA0848/NA0606 6-Phosphate Dehydrogenase(G6PDH)Activity Assay Kit

NA0835/NA0593 Isocitrate Dehydrogenase Cytoplasmic(ICDHc) Assay Kit

NA0721/NA0479 6-phosphogluconate Dehydrogenase(6-PGDH)Activity Assay Kit