NADP Malic Enzyme(NADP-ME) ActivityAssay Kit

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

Detection equipment: Spectrophotometer/Microplate reader

Cat No:BC1125

Size:100T/96S

Components

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

Reagent I:20 mL×1, store at 4℃.

Reagent II:Powder×1, store at 4℃. Add 10 mL of extract solution when the solution will be used.

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

Reagent IV:Powder×1, store at -20℃. Add 500 μLof double distilled water when the solution will be used.

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

Description

MEwidely exist 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 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, ME is divided into NAD-ME (EC1.1.1.38) and NADP-ME (EC1.1.1.40).

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

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 to 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 1 mL of extract solution, homogenate on ice bath. Centrifuge at 8000 ×g for 10 minutes at 4℃, take the supernatant and keep it on ice for test.

3. Serum:

Detect directly.

II. Procedure

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 340 nm, 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	200
Working solution	90
Sample	10

Add reagents to micro quartz cuvette orderly, mix thoroughly. Record the initial absorbance A1 and absorbance A2 after 1 minute 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<0.5 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℃ 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 produce of 1 nmol of NADPH per minute every 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 catalyzesthe produce of 1 nmol of NADPH per minute every 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 of 1nmol of NADPH per minute every 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 of1 nmol of NADPH per minute every 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 of1nmol of NADPH per minute every milliliter of serum.

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

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

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

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

Vs_A:Sample volume, 0.01 mL;

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

T: Reaction time(min), 1minute;

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. CalculateΔA=A2-A1=0.8753-0.6389=0.2364, calculate the enzyme activity according to sample weight:

NADP-ME（U/g weight）=4823×ΔA÷W=4823×0.2364÷0.1=11401.572 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=0.1305-0.1081=0.0224, calculate the enzyme activity according to sample weight:

NADP-ME（U/g weight）=4823×ΔA÷W=4823×0.0224÷0.1=1080.352 U/g weight.

3.Take 10μL serum of horse to detect directly, calculateΔA=A2-A1=0.0763-0.0738=0.0025, calculate the enzyme activity according to volume of serum:

NADP-ME（U/mL）=4823×ΔA=4823×0.0025=12.0575 U/mL.

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：

BC0260/BC0265	6-Phosphate Dehydrogenase(G6PDH)Activity Assay Kit
BC0400/BC0405	Isocitrate Dehydrogenase Cytoplasmic(ICDHc) Assay Kit
BC2100/BC2105	6-phosphogluconate Dehydrogenase(6-PGDH)Activity Assay Kit