Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

CheKine™ Micro NADP Malic Enzyme (NADP-ME) Activity Assay Kit

Cat #: KTB1018 Size: 48 T/96 T

	Micro NADP Malic Enzyme (NADP-ME) Activity Assay Kit		
REF	**Cat #:** KTB1018	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Malic enzyme (ME) is widely present in the cytoplasm of microorganisms, cultured cells, animals, and plants, especially with high activity in plant tissues. ME catalyzes the reversible oxidation decarboxylation of malic acid, producing pyruvate and CO_2, as well as the reduction reaction accompanied by $NAD(P)^+$, which is a key enzyme in malic acid metabolism. ME activity is closely related to biosynthesis and antioxidant activity. Based on the specificity of coenzyme and substrate, ME can be divided into NAD-ME (EC1.1.1.38) and NADP-ME (EC1.1.1.40). NADP-ME can catalyze the reduction of $NADP^+$ to NADPH, and the rate of NADPH increase at 340 nm can reflect its activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	15 mL	30 mL	4℃
Reagent Ⅱ	1	1	-20℃, protected from light
Reagent Ⅲ	1	1	-20℃

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Thermostatic water bath, ice maker, centrifuge
- Deionized water
- Mortar or homogenizer

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent II: Prepared before use. Add 10 mL Reagent I to 48 T and 20 mL Reagent I to 96 T, dissolve thoroughly and set aside for use; Unused reagents can be stored in a dark place at -20℃ for 4 weeks to avoid repeated freeze-thaw cycles.

Reagent III: Prepared before use. Add 1.25 mL Reagent I to 48 T and 2.5 mL Reagent I to 96 T, dissolve thoroughly and set aside for use; Unused reagents can be stored at -20℃ for 4 weeks to avoid repeated freeze-thaw cycles.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 14,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.

2. Add 10 μL sample supernatant and 170 μL Reagent II to a microquartz cuvette or 96 well UV plate, mix well, incubate at 30℃ for 5 min, then add 20 μL Reagent III, immediately record the initial absorbance value A1 at 340 nm and the absorbance value A2 after 1 min after mixing, calculate ΔA=A2-A1.

Note: Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If ΔA_{Test} is less than 0.005, the reaction time can be extended to 5 or 10 min, that is, continue to record the absorbance value at 340 nm for 5 or 10 min and calculate the corresponding ΔA, divide the calculated result by the actual reaction time. If A_{Test} is greater than 0.5, the sample supernatant can be further diluted by Extraction Buffer, and the calculation result should be multiplied by the dilution multiple.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well UV plates calculation formula as below

1. Calculated by protein concentration

Active unit definition: 1 nmol NADPH is generated per min in 1 mg tissue protein reaction system is defined as a unit of enzyme activity.

1 nmol NADPH is generated per minute per g of tissue, defined as an enzyme activity unit

NADP-ME (U/mg prot)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(Cpr\times V_{Sample})\div T$**=6,431×ΔA÷Cpr**

2. Calculated by sample fresh weight

Active unit definition: 1 nmol NADPH is generated per min per g of tissue is defined as a unit of enzyme activity.

NADP-ME (U/g fresh weight)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\times V_{Sample}\div V_{Total\ Sample})\div T$**=6,431×ΔA÷W**

V_{Total}: total reaction volume, 2×10^{-4} L; ε: NADH molar extinction coefficien, 6.22×10^3 L/mol/cm; d: 96-well plate diameter, 0.5 cm; 10^9: 1 mol=1×10^9 nmol; Cpr; sample protein concentration, mg/mL; T: reaction time, 1 min;V_{Sample}: sample volume added, 0.01 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; W: sample weight, g.

Precautions

1. During the experiment, place the sample supernatant and Reagent II on ice to prevent denaturation and inactivation.

2. The temperature of the reaction solution in the 96 well UV plate and cuvette during the experiment needs to be maintained at

30℃.

Typical Data

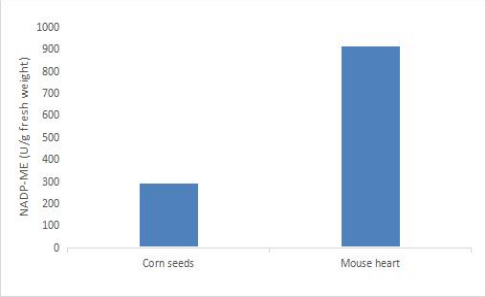

Figure 1. Determination NADP-ME activity in corn seeds and mouse heart by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.