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

Cat #: KTB3024 Size: 48 T/48 S 96 T/96 S

	Micro NAD Malic Enzyme (NAD-ME) Activity Assay Kit		
REF	**Cat #:** KTB3024	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues, Cells or Bacteria, Plasma, Serum or other Liquid samples		
	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). NAD-ME can catalyze the reduction of NAD^+ to NADH, and the rate of NADH increase at 340 nm can reflect its activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60×2 mL	4℃
Reagent Ⅰ	15 mL	30 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅲ	Powder×1 vial	Powder×1 vial	-20℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

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℃.

Working Reagent II: Prepared before use. Add 12 mL Reagent I to 48 T and 24 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.

Working 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.

1. Animal and plant tissues: 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.
2. Cells or Bacteria: Collect 10^7 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 14,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Plasma, Serum or other Liquid samples: Test directly.

Note: 1. If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

2. The animal and plant tissues samples extracted by this kit can also be used for the determination of KTB1018.

Assay Procedure

1. Preheat the microplate reader or ultraviolet 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 Working Reagent II to a microquartz cuvette or 96 well UV plate, mix well, incubate at 30℃ for 5 min, then add 20 μL Working Reagent II, immediately record the initial absorbance value A_1 at 340 nm and the absorbance value A_2 after 1 min after mixing, calculate $\Delta A=A_2-A_1$.

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 NADH is generated per min in 1 mg tissue protein reaction system is defined as a unit of enzyme activity.

NAD-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.

NAD-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**

3. Calculated by cells or bacteria number

Active unit definition: 1 nmol NADPH is generated per min per 10^4 of cells or bacteria is defined as a unit of enzyme activity.

NAD-ME (U/10^4)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(1,000×V_{Sample}÷$V_{Total\ Sample}$)÷T=**6.431×ΔA**

4. Calculated by liquid volume

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

NAD-ME (U/mL)=[ΔA×V_{Total}÷(ε×d)×10^9]÷V_{Sample}÷T=**6,431×ΔA**

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; 1,000: Total number of cells or bacteria, 10^7.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

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

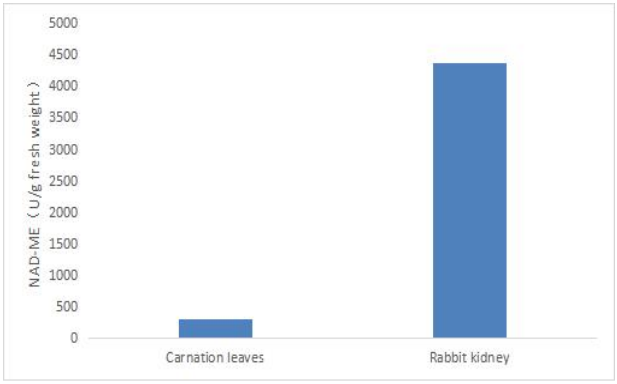

Figure 1. Determination NAD-ME activity in carnation leaves and rabbit kidney by this assay kit

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. For your safety and health, please wear a lab coat and disposable gloves.