CheKine™ Micro Pyruvate Phosphate Dikinase (PPDK) Activity Assay Kit

Cat #: KTB1129 Size: 48 T/48 S 96 T/96 S

	Micro Pyruvate Phosphate Dikinase (PPDK) Activity Assay Kit		
REF	**Cat #:** KTB1129	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant Tissues		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Pyruvate Phosphate Dikinase (PPDK, EC 2.7.9.1) is the rate-limiting enzyme in the C4 pathway and the crassulacean acid metabolism (CAM) pathway. It catalyzes the conversion of ATP, pyruvate, and inorganic phosphate (Pi) into phosphoenolpyruvate (PEP) through a three-step reaction. This enzyme is primarily found in the chloroplast stroma of C4 plants and plays a crucial role in regulating photosynthetic functions. CheKine™ Micro Pyruvate Phosphate Dikinase (PPDK) Activity Assay Kit provides a simple, convenient, and rapid method for measuring PPDK activity in plant tissue samples. The principle of the assay is based on the reverse reaction of PPDK, which catalyzes the conversion of PEP, AMP, and PPi into pyruvate, ATP, and Pi. Lactate dehydrogenase (LDH) further catalyzes the conversion of pyruvate and NADH into lactate and NAD^+. The decrease in NADH absorbance at 340 nm is measured to calculate the PPDK 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	13 mL	26 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×2 vials	-20℃, protected from light
Reagent Ⅲ	Powder×1 vial	Powder×2 vials	4℃, protected from light

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, analytical balance, ice maker, low-temperature 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. Take one bottle of Reagent Ⅱ and add 12 mL of Reagent Ⅰ and one bottle of Reagent Ⅲ. Mix thoroughly. Unused reagents should be aliquoted and stored at -20℃, protected from light, for up to 4 weeks. Avoid repeated freeze-thaw cycles.

Note: Reagent Ⅱ is toxic and has a pungent odor, so it is recommended to experiment in a fume hood.

Reagent Ⅲ: Ready to use as supplied. Equilibrate to room temperature before use. Due to the small volume, if it adheres to the tube walls, centrifuge at low speed before use to collect the liquid. Store at 4℃, protected from light.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for 2 weeks. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Note: 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.

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. According to the experimental amount needed, place Working Reagent Ⅱ in a 37℃ water bath for 5 min.

2. Add 10 μL sample supernatant and 190 μL Working Reagent Ⅱ to a microquartz cuvette or 96 well UV plate, mix well, immediately record the initial absorbance value A_1 at 340 nm and the absorbance value A_2 after 10 min after mixing, calculate $\Delta A=A_1-A_2$.

Note: Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If ΔA is less than 0.03, increase the sample volume or appropriately extend the reaction time, and divide the calculated result by the actual reaction time. If ΔA is greater than 0.5, further dilute the sample supernatant with deionized water, and multiply the calculated result by the dilution factor.

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: the consumption of 1 nmol of NADH per min per mg of tissue protein is defined as a unit of enzyme activity.

PPDK (U/mg prot)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div(Cpr\times V_{Sample})\div T$**=643×ΔA÷Cpr**

2. Calculated by sample fresh weight

Active unit definition: the consumption of 1 nmol of NADH per min per g of tissue is defined as a unit of enzyme activity.

PPDK (U/g fresh weight)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div(W\times V_{Sample}\div V_{Total\ Sample})\div T$**=643×ΔA÷W**

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

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 Working Reagent II on ice to prevent denaturation and inactivation.

Typical Data

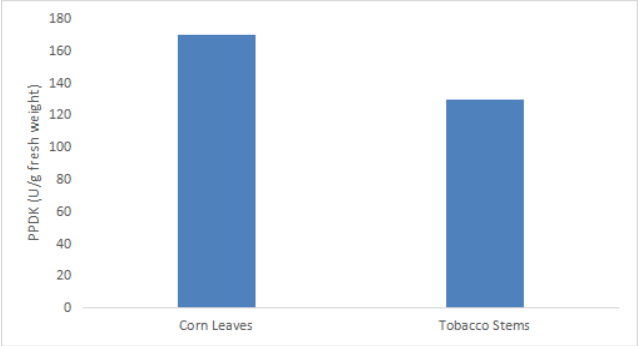

Figure 1. Determination PPDK activity in Corn Leaves and Tobacco Stems by this assay kit

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.