CheKine™ Mirco Pyrophosphate: Fructose-6-Phosphate-1-Phosphoric Acid Transferase (PFP) Activity Assay Kit

Cat #: KTB1334 Size: 48 T/96 T

	Mirco Pyrophosphate: Fructose-6-Phosphate-1-Phosphoric Acid Transferase (PFP) Activity Assay Kit		
REF	**Cat #:** KTB1334	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues, Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Phosphofructokinase (PFP, EC 2.7.1.90) is a cytoplasmic enzyme that is widely present in plant tissues. It catalyzes the reversible conversion between fructose-6-phosphate and fructose-1,6-bisphosphate and plays a crucial role in carbon metabolism during photosynthesis. CheKine™ Mirco Pyrophosphate: Fructose-6-Phosphate-1-Phosphoric Acid Transferase (PFP) Activity Assay Kit provides a simple, convenient, and rapid method for measuring PFP activity in animal and plant tissues, cells, serum (plasma), or other liquid samples. The principle of the assay is based on PFP catalyzing the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate, which is then converted to glyceraldehyde-3-phosphate by aldolase and triose phosphate isomerase. Subsequently, glyceraldehyde-3-phosphate dehydrogenase and NADH catalyze the formation of 3-phosphoglycerate, NAD, and phosphate. The change in absorbance at 340 nm reflects the activity of PFP.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	6 mL	12 mL	4℃, protected from light
Reagent Ⅱ	1	1	-20℃, protected from light
Reagent Ⅲ	1	1	-20℃, protected from light
Reagent Ⅳ	0.5 mL	1 mL	4℃, protected from light
Reagent Ⅴ	0.5 mL	1 mL	4℃, protected from light
Reagent Ⅵ	1 mL	2 mL	4℃, protected from light

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℃, protected from light.

Working Reagent II: Prepared before use. Add 1 mL deionized water to 48 T and 2 mL deionized water to 96 T, dissolve thoroughly and set aside for use; Unused reagents can be stored in a dark place at -20℃ for 2 weeks to avoid repeated freeze-thaw cycles.

Working Reagent III: Prepared before use. Add 1 mL deionized water to 48 T and 2 mL deionized water to 96 T, dissolve thoroughly and set aside for use; Unused reagents can be stored in a dark place at -20℃ for 2 weeks to avoid repeated freeze-thaw cycles.

Reagent IV: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Reagent V: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Reagent VI: Ready to use as supplied. Equilibrate to room temperature before use. 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 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 tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells: Collect 10^7 cells into the centrifuge tube, wash cells or with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 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: It will be better to quantify the total protein with Protein Quantification Kit (Bradford Assay), Cat #: KTD3002, if it is calculated by protein concentration.

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. Take a microquartz cuvette or a 96-well UV plate, and sequentially add 100 μL of Reagent Ⅰ, 20 μL of Reagent Ⅱ, 20 μL of Reagent Ⅲ, 10 μL of Reagent Ⅳ, 10 μL of Reagent Ⅴ, 20 μL of Reagent Ⅵ, and 20 μL of crude enzyme solution. Mix thoroughly and record the initial absorbance value (A_1) at 340 nm and the absorbance value (A_2) after 15 min. Calculate $\Delta A=A_1-A_2$.

Note: You can also prepare a working solution by mixing Reagent Ⅰ, Reagent Ⅱ, Reagent Ⅲ, Reagent Ⅳ, Reagent Ⅴ, and Reagent Ⅵ in the above proportions for immediate use. Do not test too many samples at once to avoid excessive delays in the enzymatic reaction time. If ΔA is less than 0.01, 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.

PFP (U/mg prot)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(Cpr×V_{Sample})÷T**=117.89×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.

PFP (U/g fresh weight)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(W×V_{Sample}÷$V_{Total\ Sample}$)÷T**=117.89×ΔA÷W**

3. Calculated by cells number

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

PFP (U/10^4)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(N×V_{Sample}÷$V_{Total\ Sample}$)÷T**=117.89×ΔA÷N**

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.

PFP (U/mL)=[ΔA×V_{Total}÷(ε×d)×10^9]÷V_{Sample}÷T**=117.89×ΔA**

V_{Total}: Total reaction volume, 2.2×10^{-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, 15 min; V_{Sample}: Sample volume added, 0.04 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; W: Sample weight, g; N: Total number of cells, 10^4.

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, keep both the sample supernatant and reagents on ice to prevent denaturation and inactivation.

Typical Data

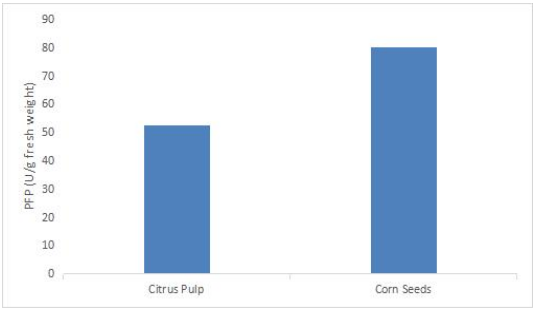

Figure 1. Determination PFP activity in Citrus Pulp and Corn Seeds by this assay kit

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.