CheKine™ Micro 6-Phosphofructokinase (PFK) Activity Assay Kit

Cat #: KTB1124 Size: 48 T/96 T

	Micro 6-Phosphofructokinase (PFK) Activity Assay Kit		
REF	**Cat #:** KTB1124	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Cell culture supernatant, Bacteria		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Phosphofructokinase (PFK, EC 2.7.1.11) is widely present in animals, plants, microorganisms and cultured cells. It is important in regulating the process of fermentation, by which one molecule of the simple sugar glucose is broken down to two molecules of pyruvic acid. The enzyme, one of a class called transferases, catalyzes one of several specific reactions involved in this breakdown—the formation of fructose-1,6-diphosphate and adenosine diphosphate (ADP) from fructose-6-phosphate and adenosine triphosphate (ATP); its activity is sensitive to the ATP/ADP ratio in the cell. PFK is one of the key regulatory enzymes in the glycolysis process. CheKine™ Micro 6-Phosphofructokinase (PFK) Activity Assay Kit provides a convenient tool for sensitive detection of PFK Activity. The principle is that PFK catalyzes fructose-6-phosphate and ATP to produce fructose-1,6-diphosphate and ADP, and pyruvate kinase and lactate dehydrogenase further catalyze the oxidation of NADH to NAD+. NADH has a maximum absorption peak detected at about 340 nm. The enzyme activity of PFK was calculated by detecting the rate of decrease in absorption at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Assay Buffer	10 mL	20 mL	4℃
Substrate Mix	1	1	4℃, protected from light
Enzyme 1	500 μL	1 mL	-20℃, protected from light
Enzyme 2	500 μL	1 mL	-20℃, 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

- Ice maker, refrigerated centrifuge, incubator
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

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

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

Substrate Mix: Add 17 mL Assay Buffer and 1.13 mL deionized water for 96 T or 8.5 mL Assay Buffer and 0.565 mL deionized water for 48 T to dissolve before use. This solution can be stored at 4℃ for 1 week. The solution can also be stored at -20℃, protected from light after aliquoting to avoid repeated freezing and thawing.

Enzyme 1: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃, protected from light.

Enzyme 2: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃, protected from light.

Sample Preparation

1. Animal tissue samples: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize. Centrifuge at 8,000 g for 10 min at 4℃. Collect the supernatant, stand by on ice, waiting for test.
2. Plant tissue samples: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Collect the supernatant, stand by on ice, waiting for test.
3. Cell or bacteria: Collect 5×10^6 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 in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Collect the supernatant, stand by on ice, waiting for test.
4. Plasma, serum or other liquid samples: Tested directly.

Note: For animal tissues with high fat content, remove the upper layer of fat after centrifugation, and then take the supernatant. It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheated 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, 10 μL Enzyme 1, 10 μL Enzyme 2 and 170 μL dissolved Substrate Mix to the 96-well UV plate or microquartz cuvette, then tap the plate and mix well. Immediately read the initial 340 nm absorbance value A_1 at 20 s, and then read the absorbance value A_2 at 10 min 20 s, and calculate $\Delta A=A_1-A_2$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the sample's ΔA_{Test}>0.5, please further dilute the sample with Extraction Buffer, or shorten the reaction time to 2 min or 5 min, bring about ΔA<0.5. Pay attention to multiply by the dilution factor or change reaction time when calculating the result.

DATA Analysis

A. 96-well UV plates calculation formula

1. Calculated by fresh weight of samples

Unit definition: 1 nmol fructose-6-phosphate and 1 nmol ATP transform into 1 nmol fructose-1,6-diphosphate and 1 nmol ADP per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

PFK (U/g)=$[\Delta A\times V_{Reaction\ Total}\div(\varepsilon\times d)\times10^9]\div(V_{Sample}\div V_{Sample\ Total}\times W)\div T\times n$**=642×ΔA÷W×n**

2. By protein concentration

Unit definition: 1 nmol fructose-6-phosphate and 1 nmol ATP transform into 1 nmol fructose-1,6-diphosphate and 1 nmol ADP per min in 1 mg tissue protein reaction system is defined as a unit of enzyme activity.

PFK (U/mg prot)=[ΔA×$V_{Reaction\ Total}$÷(ε×d)×10^9]÷(Cpr×V_{Sample})÷T×n**=642×ΔA÷Cpr×n**

3. Calculated by bacteria or cells number

Unit definition: 1 nmol fructose-6-phosphate and 1 nmol ATP transform into 1 nmol fructose-1,6-diphosphate and 1 nmol ADP per min in 10^4 bacteria or cells reaction system is defined as a unit of enzyme activity.

PFK ($U/10^4$)=[ΔA×$V_{Reaction\ Total}$÷(ε×d)×10^9]÷(V_{Sample}÷$V_{Sample\ Total}$×500)÷T×n**=1.284×ΔA×n**

4. Calculated by volume of liquid samples

Unit definition: 1 nmol fructose-6-phosphate and 1 nmol ATP transform into 1 nmol fructose-1,6-diphosphate and 1 nmol ADP per min in 1 mL liquid samples reaction system is defined as a unit of enzyme activity.

PFK (U/mL)=[ΔA×$V_{Reaction\ Total}$÷(ε×d)×10^9]÷V_{Sample}÷T×n**=642×ΔA×n**

Where: $V_{Reaction\ Total}$: Total reaction volume, 2.0 ×10^{-4} L; ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; 10^9: Unit conversion factor, 1 mol=10^9 nmol; V_{sample}: Sample volume added, 0.01 mL; $V_{sample\ Total}$: Sample extract volume, 1 mL; W: Sample weight, g; T: Reaction time, 10 min; n: Dilution factor; Cpr: Sample protein concentration, mg/mL; 500: Total number of bacteria or cells, 5×10^6.

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.

Typical Data

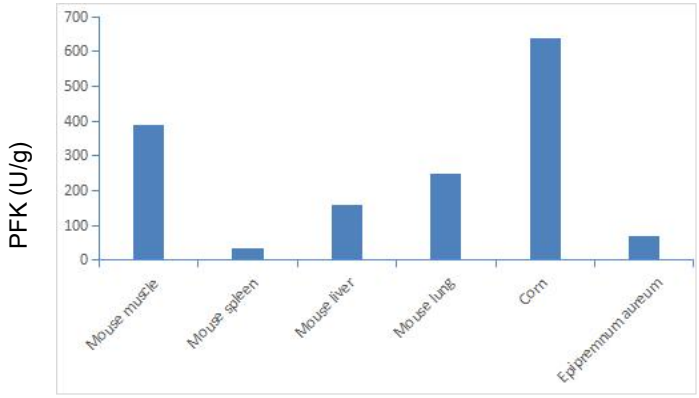

Figure 1. PFK activity in mouse muscle, mouse spleen, mouse liver, mouse lung, corn and epipremnum aureum respectively. Assays were performed following kit protocol.

Recommended Products

Catalog No.	Product Name
KTB1120	CheKine™ Micro Pyruvate Kinase (PK) Assay Kit
KTB1122	CheKine™ Micro Phosphoenolpyruvate Carboxylase (PEPC) Activity Assay Kit
KTB1121	CheKine™ Micro Pyruvate Acid (PA) Assay Kit
KTB1110	CheKine™ Micro Lactate Dehydrogenase (LDH) Assay Kit
KTB1540	CheKine™ Micro Plant Total Phenols (TP) Assay Kit

Disclaimer

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