6-Phosphofructokinase (PFK) Activity Colorimetric Assay Kit

Cat#: orb1173182 (User Manual)

6-Phosphofructokinase (PFK) Activity Colorimetric Assay Kit
Cat #: orb1173182
Lot #: Refer to product label
Applicable samples: Serum, Plasma, Animal and Plant Tissues/Cells, Cell culture supernatant, Bacterium
Storage: Stored at -20℃ for 12 months

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™ 6-Phosphofructokinase (PFK) Activity Colorimetric 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 bottle	1 bottle	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

·Standard microplate reader capable of measuring absorbance at OD340 nm
·96-well UV microplate (cat # BMB0001, Abbkine), Precision pipettes, disposable pipette tips
·Deionized water
·Dounce homogenizer

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 1.13mL 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 up to 1 week. The solution can also be stored at -20℃ 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 minutes at 4℃. Use supernatant for assay.
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 minutes at 4℃. Use supernatant for assay.
3. Cell or Bacterium: Collect 5×10^6 Cells or Bacterium into the centrifuge tube, wash Cells or Bacteriu with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the Cells or Bacterium 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 minutes at 4℃. Use supernatant for assay.
4. Plasma, Serum or other Liquid Samples: Tested directly by adding samples to the microplate.

Note: For Animal Tissues with high fat content, remove the upper layer of fat after centrifugation, and then take the supernatant. If the protein concentration of the sample is need to determined, it is recommended to use Abbkine catalog number: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheated the microplate reader for more than 30 min, and adjust the wavelength to 340 nm.
2. Add 10 µL of sample, 10 µL Enzyme 1, 10 µL Enzyme 2 and 170 µL dissolved Substrate Mix to the 96-

well UV microplate (Cat # BMB0001, Abbkine), then tap the plate and mix well. Immediately read the initial 340nm absorbance value A1 at 20 s, and then read the absorbance value A2 at 10 min 20 s, and calculate ΔA=A1-A2.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the sample's ΔATest>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

Calculate the Activity of PFK in sample

(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)=[ΔA×VReaction Total÷(ε×d)×10^9]÷(VSample÷VSample Total×W)÷T×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×VSample)÷T×n**=642×ΔA÷Cpr×n**

(3) Calculated by bacteria or cell 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 Bacterium or Cells reaction system is defined as a unit of enzyme activity.

PFK (U/10^4 cell)=[ΔA×VReaction Total÷(ε×d)×10^9]÷(VSample÷VSample Total×500)÷T×n**=1.284×ΔA×n**

(4) Calculated by volume of Liquid Samples

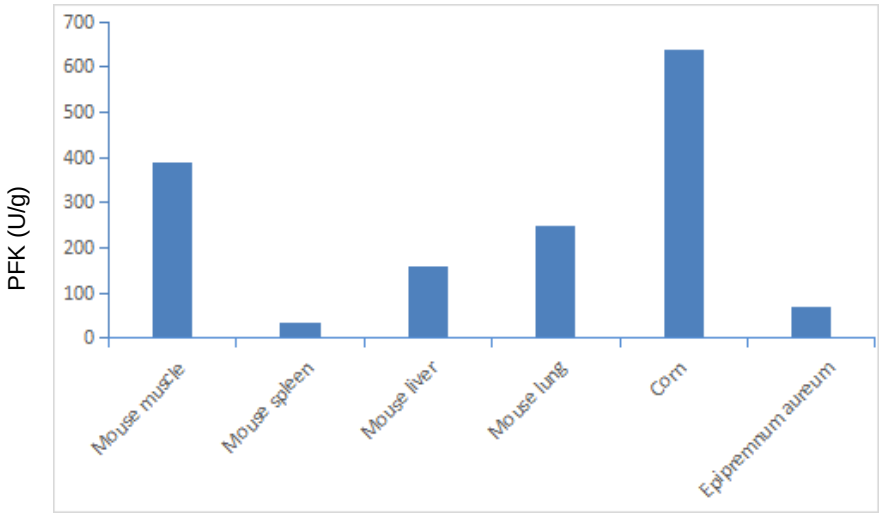
Unit Definition: 1 nmol fructose-6-phosphate and 1 nmol ATP transform into 1 nmol fructose-1,6-diphosphate and 1 nmol ADP per min in 1 mL Liquid Samples reaction system is defined as a unit of enzyme activity.

PFK (U/mL)=[ΔA×VReaction Total÷(ε×d)×10^9] ÷VSample÷T×n**=642×ΔA×n**

Where: VReaction Total: Total reaction volume, 2.0 ×10^{-4} L; ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: 96-well plate diameter, 0.5 cm; 10^9: Unit conversion factor, 1 mol=10^9 nmol; Vsample: Sample volume added, 0.01 mL; Vsample 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.

Typical data



Examples

Figure 1. PFK Activity in Mouse muscle, Mouse spleen, Mouse liver, Mouse lung, Corn and Epipremnum aureum respectively. Assays were performed following kit protocol.