Phosphofructokinase (PFK) Activity Assay Kit

Cat. No. KITG-090 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Phosphofructokinase (PFK) catalyzes the conversion of fructose-6-phosphate to fructose diphosphate, which is the second phosphorylation reaction in the glycolytic pathway. As the main rate-limiting enzyme and major regulatory point in glycolysis, the rate of glycolysis mainly depends on PFK activity. PFK activity measurement is of great significance for glycolytic flux analysis, clinical medicine, and signal transduction mechanism research. PFK catalyzes fructose-6-phosphate and ATP to produce fructose-1,6-bisphosphate and ADP. Pyruvate kinase and lactate dehydrogenase sequentially catalyze the oxidation of NADH to NAD^+. NADH has a characteristic absorption peak at 340 nm, and PFK activity can be characterized by the rate of decrease in absorbance.
Description	Detection method: Ultraviolet spectrophotometry. Detection wavelength: 340 nm. Assay principle: PFK catalyzes fructose-6-phosphate and ATP to produce fructose-1,6-bisphosphate and ADP. Pyruvate kinase and lactate dehydrogenase sequentially catalyze the oxidation of NADH to NAD^+. PFK activity is determined from the decrease in absorbance at 340 nm. Compatible sample types: Bacteria, cells, tissue, serum or plasma, culture medium, and other liquid samples. Size: 60T/50S.
Form	Kit
Applications	Glycolytic flux analysis, clinical medicine, and signal transduction mechanism research.

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Notes	Materials Required but Not Supplied UV spectrophotometer; 1 mL quartz cuvette with a 10 mm path length; mortar or homogenizer; adjustable pipette; benchtop centrifuge; water bath or incubator; and distilled water. Handling and Technical Notes 1. During the assay, Reagent III, Reagent IV, and the crude enzyme solution should be kept on ice to avoid inactivation. 2. Complete the absorbance measurements accurately at the specified time points to ensure the accuracy of the experimental results. 3. If ΔA ≥ 0.5, dilute the crude enzyme solution appropriately with Extraction Solution before the assay, or shorten the reaction time appropriately so that ΔA < 0.5 to improve detection sensitivity. Adjust the dilution factor and reaction time accordingly in the calculation. 4. To ensure accurate results and avoid reagent loss, carefully read the instructions before the assay, confirm that the reagents have been stored and prepared properly, and make sure the operating steps are clear. Perform a preliminary assay with 2-3 samples expected to show large differences. Sensitivity: Detection is based on the decrease in NADH absorbance at 340 nm; ΔA < 0.5 is recommended for optimal sensitivity.
Storage	General storage temperature: -20°C. Extraction Solution: Liquid, 60 mL × 1 bottle; store at 4°C. Reagent I: Powder × 2 vials; store at -20°C protected from light. After dissolution and aliquoting, it can be stored at -20°C for one week. Avoid repeated freeze-thaw cycles. Reagent II: Liquid, 45 mL × 1 bottle; store at 4°C. Reagent III: Liquid, 50 μL × 1 vial; store at -20°C. Reagent IV: Liquid, 30 μL × 1 vial; store at 4°C.
Reconstitution	Reagent I Preparation: Add 1.5 mL distilled water to each vial and dissolve completely before use. The dissolved reagent may be aliquoted and stored at -20°C for one week; avoid repeated freeze-thaw cycles. Reagent III Application Solution Preparation: Before use, mix Reagent III and distilled water at a volume ratio of 1:7. Prepare freshly according to the required amount. Reagent IV Application Solution Preparation: Before use, mix Reagent IV and distilled water at a volume ratio of 8:130. Prepare freshly according to the required amount. PFK Working Solution Preparation: Prepare freshly according to the required amount by mixing Reagent I

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	and Reagent II at a volume ratio of 1:15.
Reaction conditions	Sample Preparation 1. Bacteria or cells: Collect bacteria or cells by centrifugation into a centrifuge tube. Process the sample at a ratio of bacteria or cell number (10^4) to Extraction Solution volume (mL) of (500-1000):1. It is recommended to add 1 mL Extraction Solution to 10 million bacteria or cells. Sonicate in an ice bath at 20% power or 200 W using 3 s sonication and 10 s intervals, repeated 30 times. Centrifuge at 8000 × g for 10 min at 4°C. Collect the supernatant and keep it on ice for the assay. 2. Tissue: Process the sample at a ratio of tissue mass (g) to Extraction Solution volume (mL) of 1:(5-10). It is recommended to weigh 0.1 g tissue and add 1 mL Extraction Solution. Homogenize in an ice bath. Centrifuge at 8000 × g for 10 min at 4°C. Collect the supernatant and keep it on ice for the assay. 3. Serum or plasma, culture medium, and other liquid samples: Assay directly or after appropriate dilution. Assay Protocol 1. Preheat the UV spectrophotometer for more than 30 min, set the wavelength to 340 nm, and zero the instrument with distilled water. 2. Add the following reagents sequentially to a 1 mL quartz cuvette: 800 µL PFK Working Solution, 30 µL crude enzyme solution, 5 µL Reagent III application solution, and 5 µL Reagent IV application solution. 3. Mix rapidly and start timing immediately. Measure the absorbance at 340 nm at 20 s total time and record it as A_1. Allow the reaction to proceed accurately for 600 s at 37°C for mammalian samples or 25°C for samples from other species. Measure the absorbance at 340 nm at 620 s total time and record it as A_2. Calculate $\Delta A = A_1 - A_2$. Assay time: 600 s (10 min) reaction time plus instrument preheating time.
Unit definition	General Unit Definition One enzyme activity unit is defined as the amount of enzyme that catalyzes the conversion of 1 nmol fructose-6-phosphate and 1 nmol ATP to 1 nmol fructose-1,6-bisphosphate and 1 nmol ADP per minute in the reaction system, expressed per mg protein, per g tissue, per 10^4 cells, or per mL liquid sample as

applicable. 1. Calculation Based on Tissue Protein Concentration Unit definition: One enzyme activity unit is defined as the amount of enzyme that catalyzes the conversion of 1 nmol fructose-6-phosphate and 1 nmol ATP to 1 nmol fructose-1,6-bisphosphate and 1 nmol ADP per mg tissue protein per minute in the reaction system. PFK (U/mg prot) = ΔA × Vreaction total × 10^9 ÷ (ε × d × Vsample × Cpr × T) = 450 × ΔA ÷ Cpr. 2. Calculation Based on Tissue Sample Mass Unit definition: One enzyme activity unit is defined as the amount of enzyme that catalyzes the conversion of 1 nmol fructose-6-phosphate and 1 nmol ATP to 1 nmol fructose-1,6-bisphosphate and 1 nmol ADP per g tissue per minute in the reaction system. PFK (U/g) = ΔA × Vreaction total × Vsample total × 10^9 ÷ (ε × d × Vsample × W × T) = 450 × ΔA ÷ W. 3. Calculation Based on Bacteria or Cell Number Unit definition: One enzyme activity unit is defined as the amount of enzyme that catalyzes the conversion of 1 nmol fructose-6-phosphate and 1 nmol ATP to 1 nmol fructose-1,6-bisphosphate and 1 nmol ADP per 10^4 bacteria or cells per minute in the reaction system. PFK (U/10^4 cells) = ΔA × Vreaction total × Vsample total × 10^9 ÷ (ε × d × Vsample × bacteria or cell number × T) = 450 × ΔA ÷ bacteria or cell number. 4. Calculation Based on Liquid Sample Volume Unit definition: One enzyme activity unit is defined as the amount of enzyme that catalyzes the conversion of 1 nmol fructose-6-phosphate and 1 nmol ATP to 1 nmol fructose-1,6-bisphosphate and 1 nmol ADP per mL liquid sample per minute in the reaction system. PFK (U/mL) = ΔA × Vreaction total × 10^9 ÷ (ε × d × Vsample × T) = 450 × ΔA. Where: Vsample is the volume of crude enzyme solution added to the reaction system, 30 μL = 0.03 mL; Vsample total is the total volume of crude enzyme solution, 1 mL; Vreaction total is the total reaction-system volume, 8.4 × 10^{-4} L; ε is the molar extinction coefficient of NADH, 6.22 × 10^3 L/mol/cm; d is the path length of the 1 mL quartz cuvette, 1 cm; T is the reaction time, 600 s = 10 min; W is the sample mass, g; Cpr is the sample protein concentration, mg/mL; bacteria or cell number is expressed in units of 10^4; and 10^9 is the unit-conversion factor, 1 mol = 10^9 nmol.

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Usage	For Research Use Only. Not for Use in Diagnostic Procedures.

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