(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Phosphofructokinase (PFK) Assay Kit

Catalog No.: BC00168

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Phosphofructokinase (PFK) Assay Kit
Detection Method	Colorimetric
Sample Type	Tissues, cells, serum, plasma, bacteria
Assay Type	Quantitative
Detection Instrument	Ultraviolet spectrophotometer (340 nm)

Product Introduction

Phosphofructokinase (PFK) is widely distributed in animals, plants, microorganisms and cultured cells. It catalyzes the conversion of fructose-6-phosphate and ATP to fructose-1,6-bisphosphate and ADP, and serves as one of the key regulatory enzymes in the glycolysis pathway.

Principle

PFK catalyzes the reaction between 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^+. The PFK activity can be determined by measuring the decreasing rate of NADH absorbance at 340 nm.

Components

No.	Components	Size	Notes
Reagent 1	Liquid	40 mL×1	Store at 4°C
Reagent 2	Powder	1 vial	Store at -20°C. Dissolve completely with 2.8 mL distilled water before use. Aliquot and store the unused reagent at -20°C; avoid repeated freeze-thaw cycles.
Reagent 3	Powder	1 vial	Store at 4°C. Dissolve thoroughly with 0.26 mL distilled water prior to use. Aliquot any leftover reagent and store at -20°C; avoid repeated freeze-thaw cycles.

Reagent 4	Powder	1 vial	Store at 4°C. Dissolve completely with 0.26 mL distilled water before use. Aliquot the remaining reagent and store at -20°C; repeated freeze-thaw cycles are prohibited.
	Extraction Solution	60 mL×1	Store at 4°C

Storage

The kit has a validity period of 6 months.

Preparation

Centrifuge, ultraviolet spectrophotometer, water bath, 1 mL quartz cuvette, pipettor, mortar, ice and distilled water

- **Sample handling**

1. Bacteria or cultured cells: Collect bacteria or cells into centrifuge tubes and discard the supernatant after centrifugation. Add extraction solution at a ratio of 500,000-1,000,000 cells per 1 mL extraction solution (recommended: 1 mL extraction solution for 10 million bacteria or cells). Lyse bacteria or cells by sonication on ice: power 20% or 200 W, sonicate for 3 s with a 10 s interval, repeat 30 times. Centrifuge the lysate at 8000 g for 10 min at 4 °C, collect the supernatant and keep it on ice for subsequent detection.
2. Tissue samples: Homogenize tissue on ice with extraction solution at a mass-to-volume ratio of 1 g tissue: 5-10 mL extraction solution (recommended: weigh approximately 0.1 g tissue and add 1 mL extraction solution). Centrifuge the homogenate at 8000 g for 10 min at 4 °C, collect the supernatant and keep it on ice for subsequent detection.
3. Serum/plasma samples: Directly test without pretreatment.

Operation process

1. Preheat the spectrophotometer for more than 30 min, adjust the wavelength to 340 nm, and zero the instrument with distilled water.
2. Preparation of working solution (sufficient for 25 samples): Thoroughly mix 19 mL Reagent 1

with 1.26 mL Reagent 2. Unused working solution can be stored at 4 °C for one week.

3. Preheat the working solution for 10 min at 37 °C (for mammalian samples) or 25 °C (for samples from other species).

	Assay tube
Working Solution (μL)	800
Test sample (μL)	30
Reagent 3 (μL)	5
Reagent 4 (μL)	5
Add the above reagents sequentially into a 1 mL quartz cuvette and mix thoroughly immediately. Start timing simultaneously when Reagent 4 is added. Record the initial absorbance A_1 at 20 seconds and the absorbance A_2 at 10 minutes and 20 seconds under a wavelength of 340 nm, then calculate $\Delta A = A_1 - A_2$.	

Notes: PFK activity varies in different homogenized tissues. Conduct a preliminary experiment using 1-2 samples before the formal test. If $\Delta A > 0.5$, the enzyme activity is excessively high. You must dilute the homogenate supernatant to an appropriate concentration with extraction solution (multiply by the corresponding dilution factor in the calculation formula), or shorten the reaction time to 2 min or 5 min to make $\Delta A < 0.5$ for improved detection sensitivity.

Calculation

1. Calculation of PFK Activity in Serum (Plasma)

Unit definition: One unit of enzyme activity 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 per mL serum (plasma).

PFK Activity (U/mL) = $\frac{\Delta A \times V_{Total}}{\varepsilon \times d} \times 10^9 \div V_{sample} \div T = 450 \times \Delta A$

2. Calculation of PFK Activity in Tissues, Bacteria or Cells

① Calculation based on tissue protein concentration

Unit definition: One unit of enzyme activity is defined as the quantity of enzyme that catalyzes the conversion of 1 nmol fructose-6-phosphate and 1 nmol ATP into 1 nmol fructose-1,6-bisphosphate and 1 nmol ADP per minute per mg tissue protein.

PFK Activity (U/mg) = $\frac{\Delta A \times V_{Total}}{\varepsilon \times d} \times 10^9 \div (Cpr \times V_{sample}) \div T = 450 \times \Delta A \div Cpr$

② Calculation based on fresh weight of samples

Unit definition: One unit of enzyme activity 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 per gram of tissue.

PFK Activity (U/mg) = $\frac{\Delta A \times V_{Total}}{\varepsilon \times d} \times 10^9 \div \left(\frac{W \times V_{sample}}{V}\right) \div T = 450 \times \Delta A \div W$

③ Calculation based on bacterial or cell density

Unit definition: One unit of enzyme activity is defined as the enzyme amount 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 per 10,000 bacteria or cells.

PFK Activity (U/mg) = $\frac{\Delta A \times V_{Total}}{\varepsilon \times d} \times 10^9 \div \left(\frac{1000 \times V_{sample}}{V}\right) \div T = 0.45 \times \Delta A$

Annotation:

T: Reaction time, 10 min

V_{Total}: Total volume of reaction system, 8.4×10^{-4} L

ε: The molar extinction coefficient of NADH at 340 nm is 6.22×10^3 L·mol^{-1}·cm^{-1}

10^9：1mol=1×10^9nmol

d: Cuvette optical path length, 1 cm

V_{sample}: Volume of sample added, 0.03 mL

V: Volume of extraction solution added, 1 mL

W: Sample mass, g

Cpr: Protein concentration of sample, mg/mL

1000: Total number of bacteria or cells, 10 million

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.