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

Catalog No：E-BC-K612-M

Specification：48T(48 samples)/96T(96 samples)

Measuring instrument：Microplate reader（330-350 nm）

Detection range：0.27-32.29 U/L

Elabscience®Phosphofructokinase（PFK）

Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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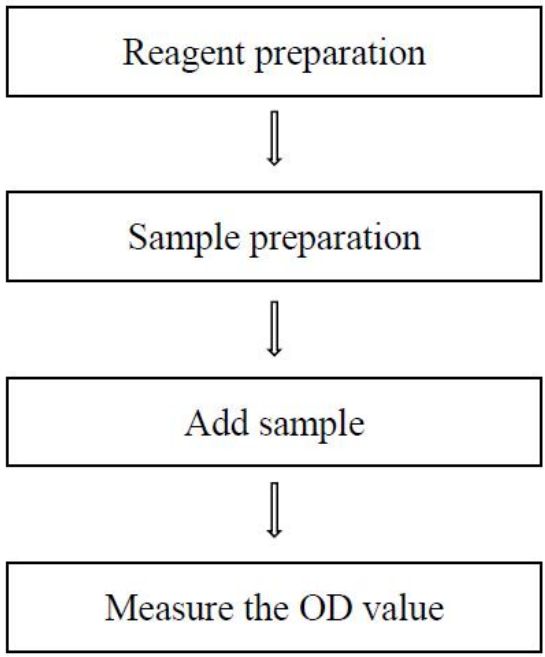
Assay summary



Intended use

This kit can be used to measure phosphofructokinase (PFK) activity in serum, plasma, tissue and cell samples.

Detection principle

Phosphofructokinase (also known as 6-phosphofructokinase; PFK) is a class of kinases that act on fructose-6-phosphate. Phosphofructokinase catalyzes fructose-6-phosphate and ATP to produce fructose-1, 6-diphosphate and ADP, then pyruvate kinase and lactate dehydrogenase further catalyze the oxidation of NADH to NAD^+. The activity of PFK can be reflected by the determination of NADH decline rate at 340 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	10 mL ×1 vial	20 mL × 1 vial	-20℃, 12 months, shading light
Reagent 2	Substrate A	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 3	Substrate B	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 4	Enzyme Reagent	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months, shading light
	UV Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Micropipettor, Vortex mixer, Incubator, Microplate reader (330-350 nm)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of working solution：

Dissolve one vial of substrate A and substrate B with 10 mL of buffer solution, mix well to dissolve. Aliquoted storage at -20°C for 3 days protected from light.

③ The preparation of substrate B working solution：

Dissolve one vial of enzyme solution with 1.2 mL of double distilled water, mix well to dissolve. Store at -20°C for 7 days protected from light.

Sample preparation

① Sample preparation

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② **Dilution of sample**

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse spleen tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse heart tissue homogenate	1
Rat serum	1
Rat plasma	1
Jurkat cell	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The working solution should be stored at -20°C with shading light and avoid repeated freeze-thaw.

② All reagents should be stored with shading light strictly.

Operating steps

① Sample well: Add 10 μL of sample to the wells.

② Add 20 μL of enzyme working solution and 170 μL of working solution into each well.

③ Measure the OD value of each well at 20 s and 5 min 20 s respectively at 340 nm with microplate reader, recorded as A_1, A_2, $\triangle A = A_1 - A_2$.

Calculation

The sample:

1. Serum (plasma) sample:

Unit definition: The enzyme amount of 1 μmol of NADH consumed by 1 L of liquid sample per minute at room temperature is defined as 1 unit.

$$\text{PFK activity (U/L)} = \frac{\Delta A_{340}}{6220 \times d} \div T \times f \times 10\text{^}6$$

2. Tissue and cells sample:

Unit definition: The enzyme amount of 1 μmol of NADH consumed by 1 g sample protein per minute at room temperature is defined as 1 unit.

$$\text{PFK activity (U/gprot)} = \frac{\Delta A_{340}}{6220 \times d} \div C_{pr} \div T \times f \times 10\text{^}6$$

[Note]

ΔA_{340}: A_2 - A_1 (A_1: the OD value at 20s; A_2: the OD value at 5 min 20 s).

6220: The molar extinction coefficient of NADH, L/mol•cm.

d: Optical path, 0.6 cm.

C_{pr}: The concentration of protein in sample, gprot/L.

T: The time of reaction, 5 min.

f: Dilution factor of sample before test.

10^6: 1 moL/L=1000000 μmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.60	18.40	26.50
%CV	3.5	3.1	2.4

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.60	18.40	26.50
%CV	7.6	8.5	7.9

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	8.5	22.3	29.5
Observed Conc. (U/L)	8.4	22.7	29.2
Recovery rate (%)	99	102	99

Sensitivity

The analytical sensitivity of the assay is 0.27 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix II Example Analysis

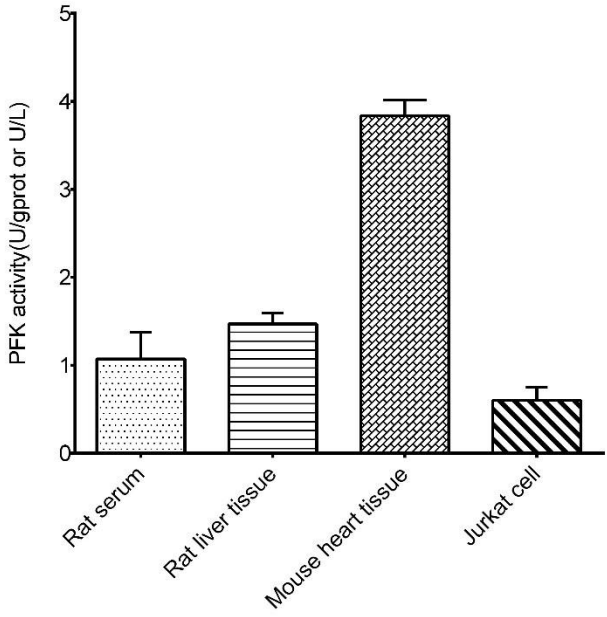
Example analysis:

For rat liver tissue, take 10 μL of 10% rat liver tissue homogenate, and carry the assay according to the operation steps. The results are as follows:

The A_1 of the sample is 1.245, after 5 minutes of reaction, the A_2 of the sample is 0.849, the concentration of protein in sample is 14.45 gprot/L, and the calculation result is:

PFK activity (U/gprot) = (1.245 – 0.849) ÷ (6220 × 0.6) ÷ 14.45 ÷ 5 × 10^6 = 1.47 U/gprot

Detect rat serum, 10% rat liver tissue homogenate (the concentration of protein is 14.45 gprot/L), 10% mouse heart tissue homogenate (the concentration of protein is 7.78 gprot/L) and Jurkat cell (the concentration of protein is 2.27 gprot/L) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.