TECHNICAL MANUAL

Phosphofructokinase (PFK) Activity Assay Kit

- **SKU CODE:** MAES0262
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Sample preparation
- Reagent preparation
- Add sample and reagents
- Incubate and measure absorbance
- Calculate PFK activity

2. Intended use

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

3. Detection principle

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

4. Kit components & storage

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

5. Materials prepared by users

Instruments:

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

Reagents:

Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of working solution:** Dissolve one vial each of Substrate A and Substrate B with 10 mL of Buffer Solution. Mix well to dissolve completely. Store in aliquots at -20 °C for up to 3 days, protected from light.
3. **Preparation of enzyme working solution:** Dissolve one vial of Enzyme Reagent with 1.2 mL of double distilled water. Mix well to dissolve completely. Store at -20 °C for up to 7 days, protected from light.

7. Sample preparation

Sample preparation:

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

4. Centrifuge at 10,000 x g for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Cell (adherent or suspension) samples:

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

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

3. Homogenize 1 x 10^6 cells in 200 µL normal saline (0.9% NaCl) using an ultrasonic cell disruptor at 4 °C.

4. Centrifuge at 10,000 x g for 10 minutes at 4 °C to remove insoluble material. Collect supernatant and keep on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

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

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 dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. Key points of the assay

1. The working solution should be stored at -20 °C, protected from light, and avoid repeated freeze-thaw cycles.
2. All reagents should be stored protected from light.

9. 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 A1 and A2. Calculate ΔA = A1 - A2.

10. Calculation

Serum (plasma) sample:

Unit definition: The enzyme amount that consumes 1 μmol of NADH per minute per liter of liquid sample at room temperature is defined as 1 unit.

PFK activity (U/L) = ΔA_340 / (6220 × d) ÷ T × f × 10^6

2. Tissue and cell sample:

Unit definition: The enzyme amount that consumes 1 μmol of NADH per minute per gram of sample protein at room temperature is defined as 1 unit.

PFK activity (U/gprot) = ΔA_340 / (6220 × d) ÷ C_{pr} ÷ T × f × 10^6

Where:

ΔA_340: A_2 - A_1 (A_1: the OD value at 20s; A_2: the OD value at 5 min 20s)

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 = 1,000,000 μmol/L

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

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.

Inter-assay Precision

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

Recovery

Three samples of high, medium, and low concentrations were tested with 6 parallel replicates for each concentration to obtain an average recovery rate of 100%.

Recovery

	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.

12. Appendix II Example Analysis

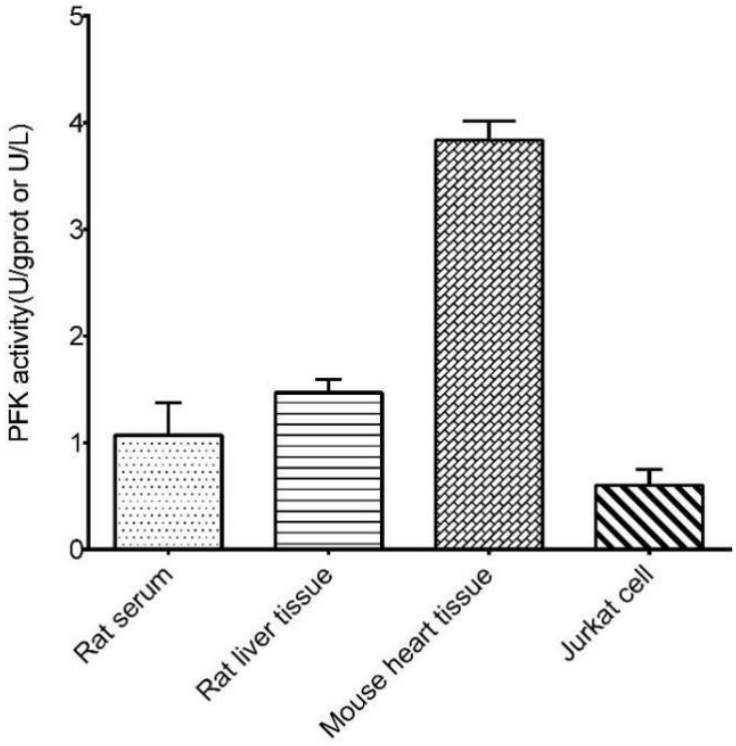
Example analysis:

For rat liver tissue, take 10 μL of 10% rat liver tissue homogenate and perform 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

Detection of rat serum, 10% rat liver tissue homogenate (protein concentration: 14.45 gprot/L), 10% mouse heart tissue homogenate (protein concentration: 7.78 gprot/L) and Jurkat cell (protein concentration: 2.27 gprot/L) according to the protocol yielded the following results:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

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Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.