

Phosphofructokinase (PFK) Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: AK0541

Size: 100T/96S

Components:

Extract solution: Liquid 100 mL ×1. Storage at 4 °C;

Reagent I: Liquid 20 mL×1. Storage at 4°C;

Reagent II : Powder ×1. Storage at -20°C; Add 17 mL of Reagent I and 1.13 mL of distilled water in Reagent II to fully dissolve, place it at 37°C (mammals) or 25°C (other species) water bath for 5 minutes. the unused reagent stored at 4°C for one week.

Reagent III : Liquid 25 μL×1. Storage at -20°C; The liquid is placed in the EP tube in the reagent bottle. Before use, according to the volume ratio of Reagent III : distilled water=1:50, fully mix, prepare when the solution will be used; it is recommended to store it separately at -20°C to avoid repeated freezing and thawing;

Reagent IV : Liquid 10 μL×1. Storage at 4°C . The liquid is placed in the EP tube in the reagent bottle. Before use, according to the volume ratio of Reagent IV : distilled water=1:125, fully mix, prepared when the solution will be used.

Product Description

PFK (EC 2.7. 1. 11) is one of the key regulatory enzymes in the process of glycolysis, which widely found in animals, plants, microorganisms and cultured cells. It is responsible for converting fructose-6-phosphate and ATP into fructose-diphosphate and ADP. PFK catalyzes the formation of fructose- 1,6-diphosphate and ADP to from fructose-6-phosphate and ATP. Pyruvate kinase and lactate dehydrogenase further catalyze the oxidation of NADH to generate NAD⁺ . The degradation rate of NADH which measured at 340 nm is used to reflect the activity of PFK.

Reagents and Equipment Required but Not Provided.

Table centrifuge, water-bath, ultraviolet spectrophotometer/microplate reader, micro quartz cuvette/96 well flat-bottom UV plate, adjustable pipette, mortar/homogenizer, ice and distilled water.

Procedure

I. Sample preparation

a. Bacteria or cells

Collect the bacteria or cells into a centrifuge tube, discard the supernatant after centrifugation. According to the addition of bacteria or cells (10⁴): Extract solution (mL) is 500- 1000:1. It is suggested that add 1 mL of Extract solution into per 5 million bacteria or cells, ultrasonic crushing bacteria or cells (power 200W,

ultrasonic of 3s, interval of 10s, repeat 30 times). Centrifuge at 8000 ×g for 10 minutes at 4°C to remove insoluble materials and take the supernatant on ice before test.

b. Tissue:

According to the addition of tissue weight (g): Extract solution (mL) is 1:5- 10. It is suggested that add 1 mL of Extract solution to 0.1 g of tissue. Homogenate on ice bath. Centrifuge at 8000 ×g for 10 minutes at 4°C to remove insoluble materials and take the supernatant on ice before test.

c. Serum (plasma) sample: Direct detection.

II. Determination

1. Preheat the ultraviolet spectrophotometer/microplate reader for more than 30 min, adjust the wavelength to 340 nm. and adjust zero with distilled water.

2. Sample test:

1) Add 10 μL of sample, 10 μL of Reagent III, 10 μL of Reagent IV and 170 μL of Reagent II in micro quartz cuvette/96 well flat-bottom UV plate, mix thoroughly, immediately record absorbance value A1 at

340 nm for 20s. After color comparison, put the cuvette or 96 well plate together with the reaction solution into a 37°C (mammal) or 25°C (other species) water bath or incubator, and react accurately for 10 minutes. Take it out and wipe it clean, immediately measure the absorbance of final reaction which record as A2(620 s). $\Delta A = A1 - A2$.

II. Calculation of PFK activity unit:

a. Calculate by micro quartz cuvette:

1. Serum (plasma):

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the conversion of 1 nmol of fructose-6-phosphate and 1 nmol of ATP into 1 nmol of fructose- 1, 6-diphosphate and 1 nmol of ADP per minute in the reaction system every milliliter of serum (plasma).

$$\text{PFK(U/mL)} = [\Delta A \times V_{TV} \div (\epsilon \times d) \times 10^9] \div V_S \div T = 321 \times \Delta A$$

2. Tissues, bacteria or cells:

1) Calculate by sample protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the conversion of 1 nmol of fructose-6-phosphate and 1 nmol of ATP into 1 nmol of fructose- 1, 6-diphosphate and 1 nmol of ADP per minute in the reaction system every milligram of tissue protein.

$$\text{PFK(U/mg prot)} = [\Delta A \times V_{TV} \div (\epsilon \times d) \times 10^9] \div (C_{pr} \times V_S) \div T = 321 \times \Delta A \div C_{pr}$$

2) Calculate by fresh weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the conversion of 1 nmol of fructose-6-phosphate and 1 nmol of ATP into 1 nmol of fructose- 1, 6-diphosphate and 1 nmol of ADP per minute in the reaction system every gram of tissue.

$$\text{PFK(U/g fresh weight)} = [\Delta A \times V_{TV} \div (\epsilon \times d) \times 10^9] \div (W \times V_S \div V_{TS}) \div T = 321 \times \Delta A \div W$$

3) Calculate by bacteria or cell density

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the conversion of 1 nmol of fructose-6-phosphate and 1 nmol of ATP into 1 nmol of fructose- 1, 6-diphosphate and 1 nmol of ADP per minute in the reaction system every 10,000 bacteria or cells.

$$\text{PFK(U/10}^4 \text{ cell)}=[\Delta A \times V_{TV} \div (\epsilon \times d) \times 10^9] \div (500 \times V_S \div V_{TS}) \div T = 0.642 \times \Delta A$$

V_{TV} : Total volume of the reaction system, 2×10^{-4} L;

ϵ : The molar extinction coefficient of NADPH is 6.22×10^3 L/mol/cm.

d : Light path of the cuvette, 1 cm;

V_S : add the sample volume, 0.01 mL;

V_{TS} : add the extract solution volume, 1 mL;

T : Reaction time, 10 min;

C_{pr} : sample protein concentration, mg/mL;

W : sample mass, g;

10^9 : Unit conversion factor, 1 mol= 10^9 nmol;

500: Total number of bacteria or cells, 5 million.

b. Calculate by 96 well flat-bottom UV plate:

1. Serum (plasma):

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the conversion of 1 nmol of fructose-6-phosphate and 1 nmol of ATP into 1 nmol of fructose- 1, 6-diphosphate and 1 nmol of ADP per minute in the reaction system every milliliter of serum (plasma).

$$\text{PFK(U/mL)}=[\Delta A \times V_{TV} \div (\epsilon \times d) \times 10^9] \div V_S \div T = 535 \times \Delta A$$

2. Tissues, bacteria or cells:

1) Calculate by sample protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the conversion of 1 nmol of fructose-6-phosphate and 1 nmol of ATP into 1 nmol of fructose- 1, 6-diphosphate and 1 nmol of ADP per minute in the reaction system every milligram of tissue protein.

$$\text{PFK(U/mg prot)}=[\Delta A \times V_{TV} \div (\epsilon \times d) \times 10^9] \div (C_{pr} \times V_S) \div T = 535 \times \Delta A \div C_{pr}$$

2) Calculate by fresh weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the conversion of 1 nmol of fructose-6-phosphate and 1 nmol of ATP into 1 nmol of fructose- 1, 6-diphosphate and 1 nmol of ADP per minute in the reaction system every gram of tissue.

$$\text{PFK (U/g fresh weight)}=[\Delta A \times V_{TV} \div (\epsilon \times d) \times 10^9] \div (W \times V_S \div V_{TS}) \div T = 535 \times \Delta A \div W$$

3) Calculate by bacterial or cell density:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the conversion of 1 nmol of fructose-6-phosphate and 1 nmol of ATP into 1 nmol of fructose- 1, 6-diphosphate and 1 nmol of ADP per minute in the reaction system every 10,000 bacteria or cells.

$$\text{PFK(U/10}^4 \text{ cell)}=[\Delta A \times V_{TV} \div (\epsilon \times d) \times 10^9] \div (500 \times V_S \div V_{TS}) \div T = 1.07 \times \Delta A$$

V_{TV} : Total volume of the reaction system, 2×10^{-4} L;

ϵ : The molar extinction coefficient of NADPH is 6.22×10^3 L/mol/cm.

d : Light path of the 96 well flat-bottom, 0.6 cm;

V_S : Sample volume, 0.01 mL;

V_{TS} : Extraction liquid volume, 1 mL;

T: Reaction time, 10 minutes;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

10^9 : Unit conversion factor, 1 mol= 10^9 nmol;

500: Total number of bacteria or cells, 5 million.

Note:

1. Reagent **III**, Reagent **IV** and sample should be placed on ice to avoid denaturation and inactivation.
2. The temperature of the reaction solution in the cuvette must be maintained at 37°C or 25°C . Take a small beaker and add in a certain amount of distilled water and must keep the temperature be at 37°C or 25°C . Place the beaker in a 37°C or 25°C water bath. In the reaction process, place the cuvette with the reaction solution in the beaker.
3. It is better for two people to do this experiment at the same time, one for measure the absorbance and one for timing to ensure the accuracy of the experimental results.
4. The activity of PFK in different homogenates is different. Please sit in 1-2 pre-experiments before the formal test. If $\Delta A > 0.5$, it means that the activity is too high. It is necessary to dilute homogenate supernatant to the appropriate concentration through using Extract solution (multiply the corresponding dilution times in the formula), or shorten the reaction time to 2 min or 5 min to make $\Delta A < 0.5$, so as to improve the sensitivity of detection.

Experimental example:

1. 0.1 g of ryegrass is added into 1 mL of Extract solution for homogenization. After the supernatant is taken out, the operation is carried out according to the determination steps. measured with a micro quartz cuvette: $\Delta A = A1-A2 = 1.6086- 1.2986 = 0.31$.

PFK (U/g mass) = $535 \times \Delta A \div W = 535 \times 0.31 \div 0.1 = 1658.5$ U/g mass.

2. Take 0.1 g of peach leaves and add 1 mL of Extract solution for homogenization. After taking the supernatant, operate according to the determination steps. Measure with micro quartz cuvette and calculate $\Delta A=A1-A2 = 1.7025- 1.6159 = 0.0866$.

PFK (U/g mass) = $535 \times \Delta A \div W = 535 \times 0.057 \div 0.1 = 463.31$ U/g mass.

References:

[1] Papagianni M, Avramidis N. Lactococcus lactis as a cell factory: a twofold increase in phosphofructokinase activity results in a proportional increase in specific rates of glucose uptake and lactate formation[J]. Enzyme and microbial technology, 2011, 49(2): 197-202.

Related Products:

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| AK0396/AK0395 | Lactic Acid(LA) Content Assay Kit |
| AK0247/AK0242 | Phosphoenolpyruvate Carboxylase(PEPC) Activity Assay Kit |
| AK0394/AK0393 | Phosphoglycerate Kinase(PGK) Activity Assay Kit |