GENLISA® 6-Phosphofructokinase, PFK Activity Assay

Cat No: KBCA1006

Pack Size: 100T / 96S

Components:

Extraction solution : 100 ml x 1 Bottle
Reagent I: 20 ml x 1 Bottle
Reagent II: Powder x 1 vial (add 17ml of reagent I and 1.13 ml of distilled water and place it in 37°C water for 5 min)
Reagent III: 25 ul x 1 Bottle
Reagent IV: 10 ul x 1 Bottle

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.

Protocol

I. Sample Preparation:

1. 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 at the intervals of 10s and repeat 30 times) centrifuge at 8000 x g for 10 mins at 4°C, collect the supernatant and keep it in ice.

2. Cell Culture / 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℃ to remove insoluble materials and take the supernatant on ice before test.

3. Serum (plasma) sample: Direct detection.

1. Reagent preparation:

- **Reagent II:** add 17ml of reagent I and 1.13 ml of distilled water and place it in 37°C water for 5 min.
- **Reagent III**: dilute it with distilled water = 1:50.

- **Reagent IV:** dilute it with distilled water = 1:125.

II. Determination procedure:

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 ul of sample, 10 ul of Reagent III , 10 ul of Reagent IV and 170 ul 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℃ (mammal) or 25℃ (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). ΔA=A1-A2.

2. Calculation Formula and Example:

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).

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

2) Tissue, Bacteria or cells:

- **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.

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

- **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.

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

- **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.

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

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).

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

2) Tissue, Bacteria or cells:

- **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.

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

- **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.

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

- **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.

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

V_{TV}: Total volume of the reaction system, 2×10^{-4} L.
ε: The molar extinction coefficient of NADPH is 6.22×103 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℃ or 25℃. Take a small beaker and add in a certain amount of distilled water and must keep the temperature be at 37℃ or 25℃. Place the beaker in a 37℃ or 25℃ 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 ΔA > 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 ΔA < 0.5, so as to improve the sensitivity of detection.

LIMITED WARRANTY

Krishgen Biosystems Private Limited does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems Private Limited, or against damages resulting from such non-Krishgen Biosystems Private Limited made products or components. Krishgen Biosystems Private Limited passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems Private Limited.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems Private Limited shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems Private Limited shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems Private Limited be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems Private Limited with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Krishgen Biosystems Private Limited. 2025

THANK YOU FOR USING A KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS PRIVATE LIMITED | OUR REAGENTS | YOUR RESEARCH |