Ver.250601

Phosphofructokinase (PFK) Activity Assay Kit

BC10003-02(100 Tests/96 Samples)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

I. Product Description

PFK 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 340nm is used to reflect the activity of PFK.

II. Reagent Composition & Preparation

Reagent	Volume	Storage
Extract Solution	110mL×1	2-8℃
Reagent I	20mL×1	2-8℃
Reagent II A	Powder ×1	-20℃
Add 1mL of distilled water and dissolve fully when the solution will be used		
Reagent II B	Powder×1	-20℃
Add 1mL of distilled water and dissolve fully when the solution will be used		
Reagent II C	Powder×2	-20℃
Add 0.5mL of distilled water for one tube and dissolve fully when the solution will be used		
Reagent II D	Powder×1	-20℃
Add 1mL of distilled water and dissolve fully when the solution will be used		
Reagent III	25μL×1	2-8℃
Mix Reagent III and distilled water in a 1μL: 50μL volume ratio (approximately for 5 tests). Mix thoroughly before use		
Reagent IV	10μL×1	2-8℃
Mix Reagent III and distilled water in a 1μL: 125μL volume ratio (approximately for 5 tests). Mix thoroughly before use		

Note:

1. Store the left reagent at -20 °C in single-use aliquots to maintain stability for up to 2-4 weeks and prevent freeze-thaw degradation.
2. Preparation of PFK Working Solution: Mix Reagent I, Reagent II A, Reagent II B, Reagent II C, and Reagent II D in a volume ratio of 8mL : 0.5mL : 0.5mL : 0.5mL : 0.5mL (sufficient for approximately 55 tests). Mix thoroughly before use.

III. Required but Not Provided

Ultraviolet spectrophotometer/Microplate reader, table centrifuge, water bath/ constant temperature incubator, adjustable pipette, micro quratz cuvette/ //96 well UV plate, mortar/homogenizer/ cell ultrasonic crusher, ice and distilled water.

IV. Protocol

I. Sample preparation

1. **Bacteria or cells:** Collect bacteria or cells into a centrifuge tube, centrifuge and discard the supernatant; The ratio of bacteria/cell amount (10^4): the volume of Extract solution (mL) is 500~1000:1 (it is suggested to take about 5 million bacteria/cells and add 1mL of Extract solution). Bacteria/cell is split by ultrasonic (placed on ice, 200W, work time 3seconds, interval 10seconds, repeat 30 times). Centrifuge at 8000 ×g for 10 minutes at 4℃, take the supernatant and placed on the ice for test.
2. **Tissue:** Add Extract solution according to the ratio of tissue mass (g): Extract solution volume(mL) = 1:5 ~10 (it is recommended to weigh 0.1g sample and add 1.0mL Extract solution),after ice bath homogenization, centrifuge at 4℃, 8000 ×g for 10min, take supernatant and placed on the ice for test.

3. **Serum (plasma) sample:** Detect sample directly. (If the solution is turbid, centrifuge to take the supernatant and then measure)

II. Determination procedure and sample list

1. Preheat ultraviolet spectrophotometer for 30minute, adjust wavelength to 340 nm, set zero with distilled water.
2. Preparation of PFK working fluid at 37℃ for 10minutes before use.
3. Operation table:

Reagent Name (μL)	Test Tube (T)
Sample	10
Reagent III	10
Reagent IV	10
PFK Working Solution	170

Add the above reagents to the micro quartz cuvette or 96-well UV plate in sequence. Detect the absorbance at 340 nm at the time of 20 seconds record as A_1 (20seconds). then place dishes with the reaction solution in a 37℃ water bath for 10 minutes (If the microplate reader has temperature control function, adjust the temperature to 37℃). Take it out and wipe it clean, immediately measure the absorbance of final reaction which record as A_2 (10minutes 20seconds). $\Delta A=A_1-A_2$.

III. Calculation for HK activity

A. Calculate by micro quartz cuvette:

1. Serum (plasma) :

Unit definition: Each milliliter of serum (plasma) catalyzes the conversion of 1nmol NADH to1nmol NAD^+ per minute in the reaction system defined as a unit of enzyme activity.

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

2. Tissues, bacteria or cells:

a) Calculate by sample protein concentration:

Unit definition: Each milligram of hiprotein catalyzes the conversion of 1nmol NADHto1nmol NAD+ per minute in the reaction system defined as a unit of enzyme activity.

PFK (U/mg prot) = $[\Delta A\times V_{rv} \div (\varepsilon\times d) \times 10^{9}] \div (Cpr\times V_s) \div T=321\times\Delta A \div Cpr$

b) Calculate by sample weight:

Unit definition: Each gram of tissue catalyzes the conversion of 1nmol NADH to 1nmol NAD^+ per minute in the reaction system defined as a unit of enzyme activity.

PFK (U/g weight)=$[\Delta A\times V_{rv}\div(\varepsilon\times d)\times 10^{9}] \div(W\times V_s\div V_e)\div T=321\times\Delta A \div W$

c) Calculate by bacteria or cell density

Unit definition: The catalytic conversion of 1 1nmol NADH to 1nmol NAD^+ per 10,000 bacteria or cells per minute in the reaction system is defined as one unit of enzyme activity.

PFK (U/10 4 cell) = $[\Delta A\times V_{rv} \div (\varepsilon\times d) \times 10^{9}] \div (N\times V_s\div V_e) \div T=321\times\Delta A\div N$

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

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

d: Light path of the cuvette, 1 cm;

V_S: Add the sample volume, 0.01mL;

V_e: Add extraction liquid volume, 1mL;

T: Reaction time, 5 minutes;

Cpr: Sample protein concentration, mg/mL;

W: Sample mass, g;

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

N: Total number of bacteria or cells, $\times 10^4$

B. Calculate by 96 well flat-bottom plate

Change d-1cm in the above formula to d-0.6cm (optical diameter of 96-well UV plate) for calculation.

Notes

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℃. Take a small beaker and add in a certain amount of distilled water and must keep the temperaturebeat37℃. Place the beaker in 37℃ 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. If $\Delta A > 0.5$, the enzyme solution needs to be diluted with enzyme extraction solution to make $\Delta A < 0.5$, which can improve the detection sensitivity. Pay attention to synchronously modifying the calculation formula.