Ver.250601

Pyruvate Kinase（PK）Activity Assay Kit

BC10002-02 (100 Tests/96 Samples)

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

I. Product Description

Pyruvate Kinase (PK) is widely present in animals, plants, microorganisms and cultured cells. It could catalyze the final step of the glycolysis process. PK is one of the major rate-limiting enzymes in the glycolysis process and one of the key enzymes for ATP production. Therefore, the determination of PK activity is of great significance.

PK catalyzes the generation of ATP and pyruvate from phosphoenolpyruvate and ADP. Lactate dehydrogenase further catalyzed NADH and pyruvate to generate lactic acid and NAD^+. The NADH degradation rate can measured at 340 nm to reflect the activity of PK.

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 1.2mL of distilled water and dissolve fully when the solution will be used.		
Reagent II B	Powder×2	-20℃
Add 0.645mL of distilled water for one tube and dissolve fully when the solution will be used		
Reagent II C	Powder×1	-20℃
Add 1.2mL of distilled water and dissolve fully when the solution will be used		
Reagent III	20μL×1	2-8℃
Before use, according to the amount of volume ratio Reagent Ⅲ: distilled water=5μL:295μL (30T), mix well, place on ice for standby, prepare when the solution will be used.		

Note:

1. Store the left reagent at -20 °C in single-use aliquots to maintain stability for up to 4 weeks and prevent freeze-thaw degradation.
2. Prepare the working solution freshly before use by thoroughly mixing Reagent I, Reagent II A, Reagent II B, and Reagent II C in a volume ratio of 750μL: 50μL : 50μL : 50μL, sufficient for 5 tests.

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 cultured cells: Collect bacteria or cells into the centrifuge tube, and discard supernatant after centrifugation. The number of bacteria or cells (10^4): the proportion of Extract solution volume (mL) is 500-1000:1 (it is recommended to add 1mL of Extract solution to5 million bacteria or cells), and ultrasonic crushing of bacteria or cells (placed on ice, 200W, work time 3seconds, interval 10seconds, repeat 30 times); Centrifuge at 8000 ×g and 4℃ for 10minutes, take the supernatant and place it on the ice for test.
2. The tissue weight (g): the ratio of Extract solution volume (mL) is 1:5-10 (take about 0.1g of tissue and add 1mL of the Extract solution), and conduct ice bath homogenate. Centrifuge at 8000 ×g and 4℃ for 10 minutes. Take the supernatant and place it 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 30 minutes, 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
Working Solution	180

The above reagents were added into a micro quartz colorimetric dish or a 96-well UV plate in sequence, immediately mixed thoroughly and then the absorbance value A_1 for 20seconds was measured at 340nm. The reagents were quickly placed at 37℃ for an accurate reaction of 2minutes (the temperature can be adjusted to 37℃ with the temperature control function of the microplate reader).Take out a quick wipe to determine the absorption value A_2 at 2minutes 20seconds. Calculation $\Delta A= A_1-A_2$.

III. Calculation for PK activity

A. Calculate by micro quartz cuvet**te:**

1. Calculation of liquid PK activity:
   Definition of unit: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of NADH in the reaction system per minute every milliliter of liquid.
   PK (U/mL) = $[\Delta A \times V_{rv} \div (\varepsilon \times d) \times 10^9] \div V_s \div T=1608\times\Delta A$
2. Calculate by the concentration of sample protein
   Definition of units: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of NADH in the reaction system per minute every milligram of tissue protein.
   PK (U/mg prot) ＝ $[\Delta A \times V_{rv} \div (\varepsilon \times d) \times 10^9] \div (Cpr \times V_s) \div T=1608\times\Delta A \div Cpr$
3. Calculate by fresh weight
   Definition of unit: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of NADH in the reaction system per minute every gram tissue.
   PK (U/g weight) = $[\Delta A \times V_{rv} \div (\varepsilon \times d) \times 10^9] \div (W \times V_s \div V_e) \div T=1608\times\Delta A \div W$
4. Calculate by the number of bacteria or cell
   Definition of units: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of NADH in the reaction system per minute every ten thousand bacteria or cells.
   PK ($U/10^4$ cell) ＝ $[\Delta A \times V_{rv} \div (\varepsilon \times d) \times 10^9] \div (N \times V_s \div V_e) \div T=1608\times\Delta A \div N$

   V_{rv}: Total volume of the reaction system, 2×10^{-4} L;
   ε: The molar extinction coefficient of NADH is 6.22×10^3 L/mol/cm.
   d: Light path of 1mL quartz cuvette, 1 cm;
   V_s: Add the sample volume, 0.01mL;
   V_e: Add the Extract solution volume, 1mL;
   T: Reaction time, 2 minutes;
   Cpr: Sample protein concentration, mg/mL;
   W: Sample weight, g;
   N: Total number of bacteria or cells, 10^4 cells.

B. Calculate by 96 well flat-bottom plate

1. Calculation of liquid PK activity: Definition of unit: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of NADH in the reaction system per minute every milliliter of liquid.
   PK (U/mL) = $[\Delta A \times V_{rv} \div (\varepsilon \times d) \times 10^9] \div V_s \div T = 2680 \times \Delta A$
2. Calculate by the concentration of sample protein
   Definition of units: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of NADH in the reaction system per minute every milligram of tissue protein.
   PK (U/mg prot) = $[\Delta A \times V_{rv} \div (\varepsilon \times d) \times 10^9] \div (Cpr \times V_s) \div T = 2680 \times \Delta A \div Cpr$
3. Calculate by fresh weight
   Definition of unit: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of NADH in the reaction system per minute every gram tissue.
   PK (U/g weight) = $[\Delta A \times V_{rv} \div (\varepsilon \times d) \times 10^9] \div (W \times V_s \div V_e) \div T = 2680 \times \Delta A \div W$
4. Calculate by the number of bacteria or cell
   Definition of units: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of NADH in the reaction system per minute every ten thousand bacteria or cells.
   PK ($U/10^4$ cell) = $[\Delta A \times V_{rv} \div (\varepsilon \times d) \times 10^9] \div (N \times V_s \div V_e) \div T = 2680 \times \Delta A \div N$

   V_{rv}: Total volume of the reaction system, 2×10^{-4} L;
   ε: The molar extinction coefficient of NADH is 6.22×10^3 L/mol/cm.
   d: Light path of 1mL quartz cuvette, 0.6 cm;
   V_s: Add the sample volume, 0.01mL;
   V_e: Add the Extract solution volume, 1mL;
   T: Reaction time, 2 minutes;
   Cpr: Sample protein concentration, mg/mL;
   W: Sample weight, g;
   N: Total number of bacteria or cells, 10^4 cell.

Notes

1. During the determination process, Reagent III and samples are placed on the ice to avoid denaturation and inactivation.
2. Keep the temperature of reaction solution in micro quartz cuvette at 37℃, take a small beaker, add in a certain amount of distilled water (the temperature of distilled water at 37℃), and put the beaker in 37℃ water bath pot. In the reaction process, the cuvette and the reaction solution are placed in the beaker. Or incubate enzyme label plate in 37℃ in the constant temperature incubator.
3. It is better for two people to do this experiment at the same time, one for colorimetric and the other for timing to ensure the accuracy of the experimental results.