Pyruvate Kinase（PK）Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: NA0826

Size:50T/48S

Components:

Extract solution: 60 mL ×1, Storage at 4℃.

Solution I: 45 mL×1, Storage at 4℃.

Solution II: Powder×1. Storage at -20℃.

Solution III: Powder×1. Storage at -20℃. Add 1.5 mL of distilled water to each tube and dissolve fully when the solution will be used. The left reagent can still store at 4℃ for one week.

Solution IV: Liquid 50 μL×1. Storage at 4℃. The liquid is placed in the EP tube in the reagent bottle. Before use, according to the amount of volume ratio Solution III: distilled water=1:20, mix well, place on ice for standby, prepare when the solution will be used;

Product Description

Pyruvate Kinase (PK, EC 2.7.1.40) is widely exists 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.

Reagents and Equipment Required but Not Provided

Spectrophotometer, table centrifuge, water-bath, adjustable pipette, 1 mL quartz cuvette, mortar, ice and distilled water.

Procedure

I. Sample pretreatment

a. 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 1 mL of Extract solution to 5 million bacteria or cells), and ultrasonic crushing of bacteria or cells (ice bath, 20% power or 200W, ultrasonic of 3s, 10s of interval, repeat for 30 times); Centrifuge at 8000 ×g and 4℃ for 10 minutes. The supernatant is take and placed on the ice for test.

b. Tissue:

The tissue mass (g): the ratio of Extract solution volume (mL) is 1:5-10 (take about 0.1 g of tissue and add 1 mL of the Extract solution), and conduct ice bath homogenate. Centrifuge at 8000g and 4℃ for 10 minutes. The supernatant is take and placed on the ice for test.

c. Serum (plasma) sample: Direct detection

II. Determination procedure and sample list

a. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 340 nm and set zero with distilled water.

b. Preparation of working solution: transfer Reagent II to Reagent I before use and fully dissolve it for use. Dilute it when it will be used.

c. Preheat working solution and Reagent III at 37℃ (mammals) or 25℃ (other species) for 10 minutes.

d. Sample list:

Reagent Name （μL）	Test Tube
Working solution	900
Reagent III	30
Reagent IV	15
Sample	30

Add the reagents to 1 mL quartz cuvette in order, immediately mix thoroughly, and start timing while adding the sample. Record the initial absorbance A1 at the 340 nm wavelength at 20 seconds, rapidly put cuvette together with the reaction solution in 37℃ (mammals) or 25℃ (other species) after colorimetric and react accurately in water bath for 2 minutes. Quickly remove the cuvette and dry, colorimetric at 340 nm and record the absorbance of A2 at the time of 140 seconds, calculate ΔA = A1-A2.

III. Calculation of PK vitality unit

1. Calculation of serum (plasma) 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 serum (plasma).

$PK(U/mL) = [\Delta A \times V_{TV} \div (\varepsilon \times d) \times 10^9] \div V_S \div T = 2613 \times \Delta A$

2. Calculation of PK activity in tissues, bacteria or cells:

(1) 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.

$PK(U/mg\ prot) = [\Delta A \times V_{TV} \div (\varepsilon \times d) \times 10^9] \div (Cpr \times V_S) \div T = 2613 \times \Delta A \div Cpr$

(2) 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.

$PK\ (U/g\ fresh\ weight) = [\Delta A \times V_{TV} \div (\varepsilon \times d) \times 10^9] \div (W \times V_S \div V_{TS}) \div T = 2613 \times \Delta A \div Cpr$

(3) Calculate by bacteria or cell density

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.

$PK(U/10^4\ cell) = [\Delta A \times V_{TV} \div (\varepsilon \times d) \times 10^9] \div (500 \times V_S \div V_{TS}) \div T = 5.226 \times \Delta A$

V_{TV}: total volume of the reaction system, 9.75×10^{-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, 0.03 mL;
V_{TS}: Add the extract solution volume,1 mL;
T: Reaction time, 2 minutes;
Cpr: Sample protein concentration, mg/mL;
W: Sample mass, g;
500: Total number of bacteria or cells, 5 million.

Notes

1. During the determination process, Reagent IV and samples are placed on the ice to avoid denaturation and inactivation.
2. Keep the temperature of reaction solution in cuvette at 37℃ or 25℃, take a small beaker at 37℃ and 25℃ and add in a certain amount of distilled water (the temperature of distilled water at 37℃ or 25℃), and put the beaker in 37℃ or 25℃ water bath. In the reaction process, the cuvette and the reaction solution are placed in the beaker.
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.

Recent Products Citations：

[1] Liu Y, Liang X, Zhang G, et al. Galangin and pinocembrin from propolis ameliorate insulin resistance in HepG2 cells via regulating Akt/mTOR signaling[J]. Evidence-Based Complementary and Alternative Medicine, 2018, 2018.

[2] Zhou F, Du J, Wang J. Albendazole inhibits HIF-1α-dependent glycolysis and VEGF expression in non-small cell lung cancer cells[J]. Molecular and cellular biochemistry, 2017, 428(1-2): 171-178.

References：

[1] Lepper T W, Oliveira E, Koch G D W, et al. Lead inhibits in vitro creatine kinase and pyruvate kinase activity in brain cortex of rats[J]. Toxicology in Vitro, 2010, 24(3): 1045-1051.

Related Products：

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NA0714/NA0473 Pyruvate(PA) Content Assay Kit
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