Pyruvate Kinase（PK）Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: NA0584

Size:100T/96S

Components:

Extract solution: 100 mL ×1. Storage at 4℃.

Reagent I: 20 mL×1. Storage at 4℃.

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

Reagent III: Liquid 20μL×1. Storage at 4℃.

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 catalyze 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/microplate reader, table centrifuge, water-bath, adjustable pipette, micro quartz cuvette/96 well flat-bottom plate (UV plate), ice, mortar/homogenizer 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 to break up bacteria or cells (ice bath, 20% power or 200W, ultrasonic of 3s, 10s of interval, repeat for 30 times). Centrifuge at 8000 ×g for 10 minutes at 4℃, take the supernatant and place it on 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 8000 ×g for 10 minutes at 4℃, take the supernatant and place it on ice for test.

c. Serum (plasma) sample:

Direct detection

II. Determination procedure and sample list:

(1) Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 340 nm, and set zero with distilled water.

(2) Determination

a. Add 17 mL of Reagent I and 1 mL of distilled water in Reagent II to dissolve fully, place at 37℃ (mammals) or 25 ℃ (other species) for water bath for 5 minutes, prepared when the solution will be used.

b. Before use, according to the dosage, the volume ratio of Reagent III: distilled water is 17:1000, and the mixture is placed on ice for standby.

c. 10 μL of sample, 10 μL of Reagent III and 180 μL of Reagent II are added in the micro quartz cuvette/96-well flat-bottom plate, mix thoroughly, and the absorbance A1 at 20s at 340 nm and the absorbance A2 at 140s are recorded immediately, and calculate ΔA=A1-A2.

III. Calculation of PK vitality unit:

A. Calculate by micro cuvette:

(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 per minute every milliliter serum (plasma).

$PK(U/mL)=[\Delta A\times V_{TV}\div(\varepsilon\times d)\times 10^9]\div V_S\div T=1608\times\Delta A$

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

a. Calculate by the concentration of sample protein

Definition of unit: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of NADH per minute every milligram tissue.

$PK(U/mg\ prot)=[\Delta A\times V_{TV}\div(\varepsilon\times d)\times 10^9]\div(W\times V_S\div V_{TS})\div T=1608\times\Delta A\div Cpr$

b. Calculate by sample 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 per minute every gram tissue.

$PK(U/g\ fresh\ weight)=[\Delta A\times V_{TV}\div(\varepsilon\times d)\times 109]\div(W\times V_S\div V_{TS})\div T=1608\times\Delta A\div W$

c. Calculate by bacteria or cell density

Definition of unit: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of NADH per minute every ten thousand bacteria or cells.

$PK(U/10^4\ cell)=[\Delta A\times V_{TV}\div(\varepsilon\times d)\times 10^9]\div(500\times V_S\div V_{TS})\div T=3.216\times\Delta A$

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

ε: The molar extinction coefficient of NADPH is 6.22 x 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 extraction liquid 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.

B. Calculate by 96 well flat-bottom plate

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 per minute every milliliter serum (plasma)

$PK(U/mL)=[\Delta A\times V_{TV}\div(\varepsilon\times d)\times 10^9]\div V_S\div T=2680\times\Delta A$

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

a. Calculate by the concentration of sample protein

Definition of unit: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of NADH per minute every milligram tissue.

$PK(U/mg\ prot)=[\Delta A\times V_{TV}\div(\varepsilon\times d)\times 10^9]\div(Cpr\times V_S)\div T=2680\times\Delta A\div Cpr$

b. Calculate by sample 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 per minute every gram tissue.

$PK(U/g\ fresh\ weight)=[\Delta A\times V_{TV}\div(\varepsilon\times d)\times 10^9]\div(W\times V_S\div V_{TS})\div T=2680\times\Delta A\div W$

c. Calculate by bacteria or cell density

Definition of unit: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of NADH per minute every ten thousand bacteria or cells.

$PK(U/10^4\ cell)=[\Delta A\times V_{TV}\div(\varepsilon\times d)\times 10^9]\div(500\times V_S\div V_{TS})\div T=5.36\times\Delta A$

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

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

d: Light path of the cuvette, 0.6 cm;

V_S: Add the sample volume, 0.01 mL;

V_{TS}: Add the extraction liquid 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 III 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, 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 pot. In the reaction process, the cuvette and the reaction solution are placed in the beaker. Or incubate enzyme label plate in 37℃ (mammals) or 25℃ (other species) 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.

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:

NA0809/NA0567	Hexokinase(HK) Activity Assay Kit
NA0714/NA0473	Pyruvate(PA) Content Assay Kit
NA0827/NA0585	Phosphofructokinase(PFK) Activity Assay Kit