Pyruvate Kinase（PK）Activity Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer

Catalog Number: BC0540

Size:50T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8℃
Reagent I	Liquid 50 mL×1	2-8℃
Reagent II A	Powder ×1	-20℃
Reagent II B	Powder×2	-20℃
Reagent III	Powder×1	-20℃
Reagent IV	Liquid 45 μL×1	2-8℃

Solution Preparation:

1. **Reagent II A:** Add 1.2 mL of distilled water and dissolve fully when the solution will be used. The left reagent can still store at -20℃ for 4 weeks. Avoid repeated freezing-thawing.
2. **Reagent II B:** Add 0.7 mL of distilled water for one tube and dissolve fully when the solution will be used. The left reagent can still store at -20℃ for 4 weeks. Avoid repeated freezing-thawing.
3. **Reagent III:** Add 1.8 mL of distilled water to one tube and dissolve fully when the solution will be used. The left reagent can be packaged and stored at -20℃ for 4 weeks to avoid repeated freezing and thawing.
4. **Reagent IV:** Before use, according to the amount of volume ratio Reagent IV: distilled water=5μL:100μL (7T), mix well, place on ice for standby, prepare when the solution will be used.
5. **Working solution:** according to the amount of volume ratio Reagent I: Reagent II A: Reagent II B= 860μL：20μL：20μL (1T), mix well, prepare when the solution will be used.

Product Description:

Pyruvate Kinase (PK, EC 2.7.1.40) 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

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com
For research use only. Do not use for clinical, diagnostic, food, cosmetic testing and other purposes.

NADH

degradation rate can measured at 340 nm to reflect the activity of PK.

Reagents and Equipment Required but Not Provided

Ultraviolet spectrophotometer, table centrifuge, water bath/constant temperature incubator, adjustable pipette, 1 mL quartz cuvette, mortar, ice and distilled water.

Procedure

I. Sample pretreatment

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 1 mL of Extract solution to 5 million bacteria or cells), and ultrasonic crushing of bacteria or cells (placed on ice, 200 W, work time 3s， interval 10s， repeat 30 times); Centrifuge at 8000 ×g and 4°C for 10 minutes, take the supernatant and place it on the ice for test.
2. **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 and 4°C 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:

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 340 nm and set spectrophotometer counter to zero with distilled water.
2. Preheat working solution at 37°C for 10 minutes.
3. Operation table:

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℃ 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 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) =[$\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9$] $\div Vs \div T = 2613 \times \Delta A$

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) = [$\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9$] $\div (Cpr \times Vs) \div T = 2613 \times \Delta A \div Cpr$

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)=[$\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9$] $\div (W \times Vs \div Ve) \div T = 2613 \times \Delta A \div W$

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) = [$\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9$] $\div (N \times Vs \div Ve) \div T = 2613 \times \Delta A \div N$

   Vrv: Total volume of the reaction system, 9.75×10^{-4} L;

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

   d: Light path of 1 mL quartz cuvette, 1 cm;

   Vs: Add the sample volume, 0.03 mL;

   Ve: Add the Extract solution volume,1 mL;

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

Experimental example:

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com
For research use only. Do not use for clinical, diagnostic, food, cosmetic testing and other purposes.

1. Weigh about 0.1g of rabbit heart, add 1mL of Extract solution, homogenize in an ice bath, 8000g, centrifuge at 4 ℃ for 10 minutes, take the supernatant and dilute it 16 times with the Extract solution before testing on ice. Afterwards, following the measurement steps, using a 1mL quartz cuvette,

   A1=0.690 A2=0.369，ΔA=A1-A2=0.321, calculate the activity of pyruvate kinase:
   PK activity (U/g weight)=2613×ΔA÷W×16 (dilution ratio)=134203.68 U/g weight
2. Take 30 μL of bovine serum for direct detection, using a 1mL quartz cuvette, A1=0.785 A2=0.658，ΔA=A1-A2=0.127, calculate the activity of pyruvate kinase:
   PK activity (U/mL)=2613×ΔA=331.851 U/mL
3. Weigh about 0.1g of green ivy leaves, add 1mL of Extract solution, homogenize in an ice bath, 8000g, centrifuge at 4 ℃ for 10 minutes, take the supernatant and place it on ice for testing. Afterwards, following the measurement steps, using a 1mL quartz cuvette, calculate A1=0.866 A2=0.853，ΔA=A1-A2=0.013， calculate the activity of pyruvate kinase to obtain:
   PK activity (U/g weight)=2613×ΔA÷W=336.69 U/g mass

Recent Products Citations：

[1] Xu N, Yao Z, Shang G, Ye D, Wang H, Zhang H, Qu Y, Xu F, Wang Y, Qin Z, Zhu J, Zhang F, Feng J, Tian S, Liu Y, Zhao J, Hou J, Guo J, Hou Y, Ding C. Integrated proteogenomic characterization of urothelial carcinoma of the bladder. J Hematol Oncol. 2022 Jun 3;15(1):76. doi: 10.1186/s13045-022-01291-7. PMID: 35659036; PMCID: PMC9164575.

[2] Du B, Ding D, Ma C, Guo W, Kang L. Locust density shapes energy metabolism and oxidative stress resulting in divergence of flight traits. Proc Natl Acad Sci U S A. 2022 Jan 4;119(1):e2115753118. doi: 10.1073/pnas.2115753118. PMID: 34969848; PMCID: PMC8740713.

[3] Hou DY, Xiao WY, Wang JQ, Yaseen M, Wang ZJ, Fei Y, Wang MD, Wang L, Wang H, Shi X, Cai MM, Feng HT, Xu W, Li LL. OGA activated glycopeptide-based nano-activator to activate PKM2 tetramerization for switching catabolic pathways and sensitizing chemotherapy resistance. Biomaterials. 2022 May;284:121523. doi: 10.1016/j.biomaterials.2022.121523. Epub 2022 Apr 12. PMID: 35462306.

[4] Ma WY, Wu QL, Wang SS, Wang HY, Ye JR, Sun HS, Feng ZP, He WB, Chu SF, Zhang Z, Chen NH. A breakdown of metabolic reprogramming in microglia induced by CKLF1 exacerbates immune tolerance in ischemic stroke. J Neuroinflammation. 2023 Apr 25;20(1):97. doi: 10.1186/s12974-023-02779-w. PMID: 37098609; PMCID: PMC10127063.

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：

BC0740/BC0745 Hexokinase (HK) Activity Assay Kit
BC2200/BC2205 Pyruvate (PA) Content Assay Kit

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com
For research use only. Do not use for clinical, diagnostic, food, cosmetic testing and other purposes.