# 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/Microplate reader

**Catalog Number:** BC0545

**Size:**100T/96S

**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 110 mL×1 | 2-8℃ |
| Reagent I | Liquid 20 mL×1 | 2-8℃ |
| Reagent II A | Powder ×1 | -20℃ |
| Reagent II B | Powder×2 | -20℃ |
| Reagent II C | Powder×1 | -20℃ |
| Reagent III | Liquid 20 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 be package and store at -20℃ for 4 weeks. Avoid repeated freezing-thawing.
2. **Reagent II B:** Add 0.645 mL of distilled water for one tube and dissolve fully when the solution will be used. The left reagent can be package and store at -20℃ for 4 weeks. Avoid repeated freezing-thawing.
3. **Reagent II C:** Add 1.2 mL of distilled water 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. **Working solution:** according to the amount of volume ratio Reagent I: Reagent II A: Reagent II B: Reagent II C = 750μL : 50μL : 50μL : 50μL (5T), mix well, prepare when the solution will be used.
5. **Reagent III:** 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.

**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 NADH

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

**Reagents and Equipment Required but Not Provided**

Ultraviolet spectrophotometer/Microplate reader, table centrifuge, water bath/constant temperature incubator, adjustable pipette, micro quartz cuvette/96 well UV plate, mortar/homogenizer/ cell ultrasonic crusher, 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℃ for 10 minutes, take the supernatant and place it on the ice for test.
2. **Tissue:** The tissue weight (g): the ratio of Extract solution volume (mL) is 1:5-10 (take about 0.1g of tissue and add 1 mL 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 the ultraviolet spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 340 nm and set spectrophotometer to zero with distilled water.
2. Preparation of PFK working fluid at 37℃ for 10min 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 A1 for 20s 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 A2 at 2minutes20s. Calculation ΔA=A1-A2.

**III. Calculation of PK activity:**

**A. Calculate by micro quartz cuvette:**

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.

   $PK(U/mL) = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div Vs \div T = 1608 \times \Delta A$

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.

   $PK(U/mg\ prot) = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div (Cpr \times Vs) \div T = 1608 \times \Delta A \div Cpr$

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.

   $PK\ (U/g\ weight) = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div (W \times Vs \div Ve) \div T = 1608 \times \Delta A \div W$

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.

   $PK(U/10^4\ cell) = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div (N \times Vs \div Ve) \div T = 1608 \times \Delta A \div N$

   Vrv: Total volume of the reaction system, $2 \times 10^{-4}$ L;

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

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

   Vs: Add the sample volume, 0.01 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.

**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.

   $PK(U/mL) = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div Vs \div T = 2680 \times \Delta A$

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) = [ΔA×Vrv÷(ε×d)×$10^9$] ÷(Cpr×Vs)÷T=2680×ΔA ÷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)=[ΔA×Vrv÷(ε×d)×$10^9$] ÷(W×Vs÷Ve)÷T=2680×ΔA ÷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) = [ΔA×Vrv÷(ε×d)×$10^9$] ÷(N×Vs÷Ve)÷T=2680×ΔA÷N

   Vrv: 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 1 mL quartz cuvette, 0.6 cm;

   Vs: Add the sample volume, 0.01 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 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°C, take a small beaker, add in a certain amount of distilled water (the temperature of distilled water at 37°C), and put the beaker in 37°C 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°C 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.

**Experimental example:**

1. Weigh about 0.1g of rat liver, add 1mL of Extract solution, homogenize in an ice bath, 8000g, centrifuge at 4 ℃ for 10 minutes, take the supernatant on ice. Afterwards, following the measurement steps, using a micro quartz cuvette, A1=0.726,

A2=0.243，ΔA=A1-A2=0.483, calculate the activity of pyruvate kinase:

PK activity (U/g weight)=2680×ΔA÷W=12944.4 U/g weight

**Recent Products Citations：**

[1] Yu Y, Tian D, Yu Y, Lu L, Shi W, Liu G. Microplastics aggravate the bioaccumulation and corresponding food safety risk of antibiotics in edible bivalves by constraining detoxification-related processes. Sci Total Environ. 2024 Jan 15;908:168436. doi: 10.1016/j.scitotenv.2023.168436. Epub 2023 Nov 8. PMID: 37949129.

[2] Huang C, Zhang D, Wang Z, Zhao Y, Blecker C, Li S, Zheng X, Chen L. Validation of protein biological markers of lamb meat quality characteristics based on the different muscle types. Food Chem. 2023 Nov 30;427:136739. doi: 10.1016/j.foodchem.2023.136739. Epub 2023 Jun 26. PMID: 37392625.

[3] Bai Y, Ren C, Hou C, Chen L, Wang Z, Li X, Zhang D. Phosphorylation and acetylation responses of glycolytic enzymes in meat to different chilling rates. Food Chem. 2023 Sep 30;421:135896. doi: 10.1016/j.foodchem.2023.135896. Epub 2023 Mar 8. PMID: 37098310.

[4] Kou TS, Wu JH, Chen XW, Peng B. Functional proteomics identify mannitol metabolism in serum resistance and therapeutic implications in Vibrio alginolyticus. Front Immunol. 2022 Oct 31;13:1010526. doi: 10.3389/fimmu.2022.1010526. PMID: 36389821; PMCID: PMC9660324.

[5] Zhang Z, Luo W, Chen G, Chen J, Lin S, Ren T, Lin Z, Zhao C, Wen H, Nie Q, Meng X, Zhang X. Chicken muscle antibody array reveals the regulations of LDHA on myoblast differentiation through energy metabolism. Int J Biol Macromol. 2024 Jan;254(Pt 1):127629. doi: 10.1016/j.ijbiomac.2023.127629. Epub 2023 Oct 25. PMID: 37890747.

**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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| BC2200/BC2205 | Pyruvate(PA) Content Assay Kit |
| BC0530/BC0535 | Phosphofructokinase(PFK) Activity Assay Kit |