3-Phosphoglycerate Kinase (PGK) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC2255

Size: 100T/96S

Components:

Extract solution: Liquid 110 mL×1, store at 2-8℃;

Reagent I: Liquid 10 mL×1, store at 2-8℃ and protect from light;

Reagent II: Powder×1, store at -20℃ and protect from light. Add 0.5 mL distilled water when the solution will be used. Mix thoroughly. The rest of reagent store at -20℃; Do not freeze and thaw repeatedly;

Reagent III: Powder×1, store at -20℃ and protect from light. Add 1 mL distilled water when the solution will be used. Mix thoroughly. The rest of reagent store at -20℃; Do not freeze and thaw repeatedly;

Reagent IV: Powder×1, store at -20℃ and protect from light. Add 1 mL distilled water when the solution will be used. Mix thoroughly. The rest of reagent store at -20℃; Do not freeze and thaw repeatedly;

Reagent V: Powder×2, store at -20℃. Provide an empty brown reagent bottle. Before use, take a Reagent V poured into an empty bottle and add 4 mL of distilled water to fully dissolve it. The remaining reagents can be packed and stored at-20 °C for 2 weeks. Do not freeze and thaw repeatedly. (The reagent is a freeze-dried reagent, and there may be a large difference in the amount of reagents observed by the naked eye or even a small amount. This phenomenon does not affect the use, and the actual quality is the same) ;

Working solution: according to the volume ratio of distilled water: Reagent I: Reagent II: Reagent III: Reagent IV: Reagent V=0.57mL：0.75mL：0.03mL：0.075 mL：0.075 mL：0.3 mL（total 1.8mL，10T） to prepare when the solution will be used. Mix thoroughly.

Product Description:

PGK is the key enzyme of glycolysis. It's also a key enzyme for organisms to survive. It widely exists in animals, plants and microorganisms. It has many biological functions, such as affecting DNA replication and repair, stimulating RNA synthesis of virus, and is widely used in drug target design.

PGK catalyzes the production of 1,3-diphosphoglyceride and ADP from 3-phosphoglycerate and ATP. 1,3-diphosphoglyceride produces 3-phosphoglyceraldehyde, NAD and phosphoric acid under the action of 3-phosphoglyceraldehyde dehydrogenase and NADH. The absorbance decreased at 340nm. It reflects the activity of 3-phosphoglycerate kinase.

Required but Not Provided:

Ultraviolet spectrophotometer/microplate reader, desk centrifuge, water-bath, transferpettor, micro quartz cuvette/96 well UV plate, mortar/homogenizer, ice and distilled water.

Protocol

I. Preparation:

1. **Tissue:** according to the tissue weight (g): the volume of the Extract solution (mL) is 1:5-10. It is suggested that add 1 mL of Extract solution to 0.1 g of tissue. Homogenate on ice. Centrifuge at 10000 g 4℃ for 10 minutes. Take the supernatant on ice for test.
2. **Cells:** according to the number of the cells (10^4): the volume of the Extract solution (mL) is 500~1000:1. It is suggested that add 1 mL of Extract solution to 5 million of cells. Breaking cells by ultrasonic wave in ice bath (power 300W, ultrasonic 3s, interval 7s, total time 3 min). Centrifuge at 10000 g 4℃ for 10 minutes. Take the supernatant on ice for test.
3. **Serum:** detect directly.

II. Determination procedure:

1. Preheat ultraviolet spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 340 nm, set the counter to zero with distilled water.
2. Working solution: according to the volume ratio of distilled water: Reagent I: Reagent II: Reagent III: Reagent IV: Reagent V = 6:10:2:1:1:4 to prepare when the solution will be used. Mix thoroughly.
3. Operation table: add the following reagents to the micro quartz cuvette/96 well UV plate respectively

Reagent Name(μL)	Blank tube (A_B)	Test tube (A_T)
Working solution	180	180
Sample		20
Distilled water	20	
The above reagents are added into the micro quartz cuvette/96 well UV plate in sequence. Mix thoroughly. Measure absorbance value A1 at 340 nm for 10s. Place in water bath or incubator at 37℃ (mammal) or 25℃ (other species) for 5 min. (if the microplate reader has temperature control function, the temperature can be adjusted to 37℃ or 25℃). Take out and dry rapidly. Measure the absorbance value A2 at 310s. $\Delta A_T=A1_T-A2_T$. $\Delta A_B=A1_B-A2_B$. $\Delta A=\Delta A_T-\Delta A_B$. Blank tube only need to test once or twice.		

III. PGK Calculation:

a. Micro quartz cuvette

1. Tissue

1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 nmol of NADH in the reaction system per minute every mg protein.

PGK (U/mg prot)=$\Delta A\div(\varepsilon\times d)\times V_{RT}\times 10^9\div(V_{SA}\times Cpr)\div T= 321.54\times\Delta A\div Cpr$

2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 nmol of NADH in the reaction system per minute every g sample.

PGK (U/g weight)=$\Delta A\div(\varepsilon\times d)\times V_{RT}\times 10^9\div(W\div V_E\times V_{SA})\div T=321.54\times\Delta A\div W$

3) Serum (plasma)

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 nmol of NADH in the reaction system per minute every mL serum(plasma).

PGK (U/mL)=ΔA÷(ε×d)×V_{RT}×10^9÷V_{SA}÷T=321.54×ΔA

4) Cells

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 nmol of NADH in the reaction system per minute every 10^4 cells.

PGK(U/10^4 cell)=ΔA÷(ε×d)×V_{RT}÷(500×V_{SA}÷V_E)÷T×10^9=0.643×ΔA

V_{RT}: Total reaction volume, 0.0002 L;

ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm;

d: Light path of cuvette, 1 cm;

V_{SA}: Sample volume, 0.02 mL;

V_E: Extract solution volume, 1 mL;

T: Reaction time, 5 minutes;

Cpr: Protein concentration, mg/mL;

W: Sample weight, g;

500: The number of the cells, 5 million;

10^9: Unit conversion factor, 1 mol=10^9 nmol.

b. 96 well UV plate

The optical diameter d=1 cm of the cuvette in the above formula is changed to 0.6 cm of the 96 well UV plate.

Note:

1. When ΔA is greater than 0.8 or A1 is less than 0.9 (96 well UV plate is when ΔA is greater than 0.5 or A1 is less than 0.6), it is suggested that the crude enzyme solution should be diluted with the extract and then determined. When ΔA is less than 0.01, the reaction time can be prolonged (10 min or 15 min) or the sample volume can be increased and then determined.
2. The blank tube is a test hole for testing the quality of each reagent component. Under normal conditions, the change shall not exceed 0.01.
3. The protein concentration of the sample needs to be determined by yourself. Because the extract contains protein (about 1 mg/mL), the protein concentration of the extract needs to be deducted when determining the protein concentration of the sample.

Experimental example:

1. Take 0.1g Chinese cabbage and add 1 mL of Extract solurion for homogenization, take the supernatant and then dilute the extract for 4 times, then operate according to the determination steps, measure with micro quartz cuvette and calculate ΔA_T =$A1_T$-$A2_T$ =1.05-0.5559 = 0.4941, ΔA_B=$A1_B$-$A2_B$ =0.9966-0.9941=0.0025, ΔA=ΔA-ΔA_B = 0.4941-0.0025 = 0.4916, and calculate the activity according to the sample mass:

PGK (U/g mass) = 321.54× ΔA ÷ W × dilution ratio = 321.54 × 0.4916 ÷ 0.1 × 4 = 6322.7626 U/g mass.

2. Take 0.1g mouse muscle and add 1mL of Extract solution for homogenization, take the supernatant and then dilute the extract 20 times, then operate according to the determination steps, measure with micro quartz cuvette and calculate, $\Delta A_T = A1_T - A2_T = 0.9327-0.4518 = 0.4809$, $\Delta A_B = A1_B - A2_B = 0.9966-0.9941 = 0.0025$, $\Delta A = \Delta A_T - \Delta A_B = 0.4809-0.0025 = 0.4784$.

PGK(U/g mass) = 321.54×ΔA ÷W×dilution ratio = 321.54 × 0.4784 ÷ 0.1 × 20 = 30764.9472 U/g mass.

3. Take 100 μL of camel serum sample and directly determine it according to the determination steps. Measure with micro quartz cuvette and calculate $\Delta A_T = A1_T - A2_T = 1.0435-0.9793 = 0.0642$, $\Delta A_B = A1_B - A2_B = 0.9966-0.9941 = 0.0025$, $\Delta A = \Delta A_T - \Delta A_B = 0.0642-0.0025 = 0.0617$.

PGK（U/mL）=321.54× Δ A=321.54×0.0617=19.8390 U/mL.

Related Products：

BC2270/BC2275	Fructose-bisphosphate aldolase(FBA) Activity Assay Kit
BC2240/BC2245	Fructose-1,6-diphosphate(FDP) Assay Kit
BC0530/BC0530	Phosphofructokinase(PFK) Activity Assay Kit