Phosphofructokinase (PFK) 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: BC0530

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 45 mL×1	2-8℃
Reagent II A	Powder ×1	-20℃
Reagent II B	Powder ×1	-20℃
Reagent II C	Powder ×2	-20℃
Reagent II D	Powder ×1	-20℃
Reagent III	Liquid 45 μL×1	2-8℃
Reagent IV	Liquid 20 μL×1	2-8℃

Solution Preparation:

1. **Reagent II A:** Add 1 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 1 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.
3. **Reagent II C:** Add 0.25 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 2 weeks. Avoid repeated freezing-thawing.
4. **Reagent II D:** Add 1 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.
5. **Reagent III:** According to the volume ratio of Reagent III: distilled water is 2μL:13μL (about 3T), mix well before use.
6. **Reagent IV:** According to the volume ratio of Reagent IV: distilled water is 4μL:65μL (about 13T), mix well before use.
7. **Preparation of PFK working solution:** According to the amount of volume ratio Reagent I: Reagent II A: Reagent II B: Reagent II C : Reagent II D = 22mL： 0.5mL： 0.5mL： 0.25mL： 0.5mL (about 29T), mix well, ready for use.

Product Description

PFK (EC 2.7.1.11) is one of the key regulatory enzymes in the process of glycolysis, which

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widely

found in animals, plants, microorganisms and cultured cells. It is responsible for converting fructose-6-phosphate and ATP into fructose-diphosphate and ADP.

PFK catalyzes the formation of fructose-1,6-diphosphate and ADP to from fructose-6-phosphate and ATP. Pyruvate kinase and lactate dehydrogenase further catalyze the oxidation of NADH to generate NAD^+. The degradation rate of NADH which measured at 340 nm is used to reflect the activity of PFK.

Reagents and Equipment Required but Not Provided.

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

Procedure

1. Sample pretreatment

1. **Bacteria or cells:** Collect bacteria or cells into a centrifuge tube, centrifuge and discard the supernatant; The ratio of bacteria/cell amount (10^4): the volume of Extract solution (mL) is 500~1000:1 (it is suggested to take about 5 million bacteria/cells and add 1 mL of Extract solution). Bacteria/cell is split by ultrasonic (placed on ice, 200 W, work time 3 s， interval 10 s， repeat 30 times). Centrifuge at 8000g for 10 minutes at 4℃, take the supernatant and placed on the ice for test.
2. **Tissue:** Add Extract solution according to the ratio of tissue mass (g): Extract solution volume (mL) = 1:5 ~10 (it is recommended to weigh 0.1g sample and add 1.0mL Extract solution), after ice bath homogenization, centrifuge at 4℃, 8000g for 10min, take supernatant and placed 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 340nm, set spectrophotometer counter to zero with distilled water.
2. Preparation of PFK working solution at 37℃ for 10min before use.
3. Operation table

Reagent Name (μL)	Test Tube (T)
PFK Working solution	800
Sample	30
Reagent III	5
Reagent IV	5

Add the above reagents to 1 mL quartz cuvette in sequence and start timing. Detect the

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absorbance at 340 nm at the time of 20 seconds record as A1(20s). then place dishes with the reaction solution in a 37℃ water bath for 10 minutes. Take it out and wipe it clean, immediately measure the absorbance of final reaction which record as A2(620s). $\Delta A=A1-A2$.

III. Calculation of PFK activity unit:

1. Serum (plasma):

   Unit definition: Each milliliter of serum (plasma) catalyzes the conversion of 1nmol NADH to 1 nmol

   NAD^+ per minute in the reaction system defined as a unit of enzyme activity.

   PFK (U/mL)=$[\Delta A\times Vrv\div(\varepsilon\times d)\times 10^9]\div Vs\div T=450\times \Delta A$

2. Tissues, bacteria or cells:

(1) Calculated by sample protein concentration

   Unit definition: Each milligram of hiprotein catalyzes the conversion of 1 nmol NADH to 1 nmol NAD^+ per minute in the reaction system defined as a unit of enzyme activity.

   PFK (U/mg prot)=$[\Delta A\times Vrv\div(\varepsilon\times d)\times 10^9]\div(Cpr\times Vs)\div T=450\times \Delta A\div Cpr$

(2) Calculated by sample weight

   Unit definition: Each gram of tissue catalyzes the conversion of 1nmol NADH to 1 nmol NAD^+ per minute in the reaction system defined as a unit of enzyme activity.

   PFK (U/g weight)=$[\Delta A\times Vrv\div(\varepsilon\times d)\times 10^9]\div(W\times Vs\div Ve)\div T=450\times \Delta A\div W$

(3) Calculate by the number of bacterial or cell

   Unit definition: The catalytic conversion of 1nmol NADH to 1 nmol NAD^+ per 10,000 bacteria or cells per minute in the reaction system is defined as one unit of enzyme activity.

   PFK (U/10^4 cell)=$[\Delta A\times Vrv\div(\varepsilon\times d)\times 10^9]\div(N\times Vs\div Ve)\div T=450\times \Delta A\div N$

   Vrv: Total reaction volume, 8.4×10^{-4} L;

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

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

   Vs: Sample volume, 0.03 mL;

   Ve: Extract solution volume, 1 mL;

   T: Reaction time, 10 minutes;

   Cpr: Sample protein concentration, mg/mL;

   W: Sample weight, g;

   N: Total number of bacteria or cells, 10^4 cell.

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

Note:

1. Reagent III, Reagent IV and sample should be placed on ice to avoid denaturation and inactivation.

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2. The temperature of the reaction solution in the cuvette must be maintained at 37℃. Take a small beaker and add in a certain amount of distilled water and must keep the temperature be at 37℃. Place the beaker in 37℃ water bath. In the reaction process, place the cuvette with the reaction solution in the beaker.
3. It is better for two people to do this experiment at the same time, one for measure the absorbance and one for timing to ensure the accuracy of the experimental results.
4. If ΔA > 0.5, the enzyme solution needs to be diluted with enzyme extraction solution to make ΔA < 0.5, which can improve the detection sensitivity. Pay attention to synchronously modifying the

   calculation formula.

Experimental example:

1. Take 0.1g of ryegrass is added into 1 mL of Extract solution for homogenization. After the supernatant is taken out, the operation is carried out according to the determination steps. measured with a micro quartz cuvette: ΔA = A1-A2 =1.375-0.995=0.380. To calculate:
   PFK (U/g weight) = 450 × Δ A ÷ W = 450×0.380 ÷0.1=1710 U/g weight.
2. Take 0.1 g of peach leaves and add 1 mL of Extract solution for homogenization. After taking the supernatant, operate according to the determination steps. Measure with a micro quartz cuvette and calculate ΔA=A1-A2 = 1.38-1.323=0.057. To calculate:
   PFK (U/g weight) = 450 × ΔA÷ W = 450×0.057 ÷0.1=256.5 U/g weight.

Recent Product Citations:

[1] Jiang Y, Cao S, Zhou B, Cao Q, Xu M, Sun T, Zhao X, Zhou Z, Wang Y. Hemocytes in blue mussel Mytilus edulis adopt different energy supply modes to cope with different BDE-47 exposures. Sci Total Environ. 2023 Aug 10;885:163766. doi: 10.1016/j.scitotenv.2023.163766. Epub 2023 May 3. PMID: 37146804.

[2] 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.

[3] Lai T, Sun Y, Liu Y, Li R, Chen Y, Zhou T. Cinnamon Oil Inhibits Penicillium expansum Growth by Disturbing the Carbohydrate Metabolic Process. J Fungi (Basel). 2021 Feb 9;7(2):123. doi: 10.3390/jof7020123. PMID: 33572180; PMCID: PMC7915993.

[4] Zhang Z, Liang W, Luo Q, Hu H, Yang K, Hu J, Chen Z, Zhu J, Feng J, Zhu Z, Chi Q, Ding G. PFKP Activation Ameliorates Foot Process Fusion in Podocytes in Diabetic Kidney Disease. Front Endocrinol (Lausanne). 2022 Jan 14;12:797025. doi: 10.3389/fendo.2021.797025. PMID: 35095764; PMCID: PMC8794994.

References:

[1] Papagianni M, Avramidis N. Lactococcus lactis as a cell factory: a twofold increase in

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phosphofructokinase activity results in a proportional increase in specific rates of glucose uptake and lactate formation[J]. Enzyme and microbial technology, 2011, 49(2): 197-202.

Related Products:

BC2200/BC2205	Pyruvate (PA) Content Assay Kit
BC2230/BC2235	Lactic Acid (LA) Content Assay Kit
BC2190/BC2195	Phosphoenolpyruvate Carboxylase (PEPC) Activity Assay Kit
BC2250/BC2255	Phosphoglycerate Kinase (PGK) Activity Assay Kit

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