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/Microplate reader

Catalog Number: BC0535

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 ×1	-20℃
Reagent II C	Powder ×2	-20℃
Reagent II D	Powder ×1	-20℃
Reagent III	Liquid 25 μL×1	2-8℃
Reagent IV	Liquid 10 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 0.5 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 1μL: 50μL (about 5T), mix well before use.
6. **Reagent IV:** According to the volume ratio of Reagent IV: distilled water is 1μL: 125μL (about 12T), 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 = 8mL：0.5mL：0.5mL：0.5mL:0.5mL (about 55T), 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 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/microplate reader, table centrifuge, water bath/constant temperature incubator, micro quartz cuvette/96 well flat-bottom UV plate, adjustable pipette, mortar/homogenizer/ cell ultrasonic crusher, ice and distilled water.

Procedure

I. Sample preparation

I. **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, 200W, work time 3s，interval 10s，repeat 30 times). Centrifuge at 8000 ×g for 10 minutes at 4℃, take the supernatant and placed on the ice for test.

II. **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℃, 8000 ×g for 10min, take supernatant and placed on the ice for test.

III. **Serum (plasma) sample:** Detect sample directly. (If the solution is turbid, centrifuge to take the supernatant and then measure)

II. Determination

1. Preheat the ultraviolet spectrophotometer/microplate reader for more than 30 min, adjust the wavelength to 340 nm. and adjust 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
Reagent IV	10
PFK Working Solution	170

Add the above reagents to the micro quartz cuvette or 96-well UV plate in sequence. Detect the 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 (If the microplate reader has temperature control function, adjust the temperature to 37℃). Take it out and wipe it clean, immediately measure the absorbance of final reaction which record as A2(10min20s). ΔA=A1-A2

II. Calculation of PFK activity unit:

a. Calculate by micro quartz cuvette:

1. Serum (plasma):

Unit definition: Each milliliter of serum (plasma) catalyzes the conversion of 1nmol NADH to 1nmol NAD^+ per minute in the reaction system defined as a unit of enzyme activity.

$PFK(U/mL)=[\Delta A\times Vrv\div(\varepsilon\times d)\times 10^9]\div Vs\div T=321\times\Delta A$

2. Tissues, bacteria or cells:

(1) Calculate by sample protein concentration:

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

$PFK(U/mg\ prot)=[\Delta A\times Vrv\div(\varepsilon\times d)\times 10^9]\div(Cpr\times Vs)\div T=321\times\Delta A\div Cpr$

(2) Calculate by sample weight:

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

$PFK(U/g\ weight)=[\Delta A\times Vrv\div(\varepsilon\times d)\times 10^9]\div(W\times Vs\div Ve)\div T=321\times\Delta A\div W$

(3) Calculate by bacteria or cell density

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

$PFK(U/10^4\ cell)=[\Delta A\times Vrv\div(\varepsilon\times d)\times 10^9]\div(N\times Vs\div Ve)\div T=321\times\Delta A\div N$

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 the cuvette, 1 cm;

Vs: add the sample volume, 0.01 mL;

Ve: add the extract solution volume, 1 mL;

T: Reaction time, 10 min;

Cpr: sample protein concentration, mg/mL;

W: sample weight, g;

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

N: Total number of bacteria or cells, $\times10^4$.

b. Calculate by 96 well flat-bottom UV plate:

Change d-1cm in the above formula to d-0.6cm (optical diameter of 96-well UV plate) for calculation.

Note:

1. Reagent III, Reagent IV and sample should be placed on ice to avoid denaturation and inactivation.
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 = 1.6086-1.2986 = 0.31. To calculate:
   PFK (U/g weight) = 535 × ΔA ÷ W = 535 × 0.31÷0.1 = 1658.5 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 = 1.7025-1.6159 = 0.0866. To calculate:
   PFK (U/g weight) = 535 × ΔA÷ W = 535 × 0.057÷0.1 = 463.31 U/g weight.

Recent Product Citations:

[1] Yu Y, Hu L, Tian D, Yu Y, Lu L, Zhang J, Huang X, Yan M, Chen L, Wu Z, Shi W, Liu G. Toxicities of polystyrene microplastics (MPs) and hexabromocyclododecane (HBCD), alone or in combination, to the hepatopancreas of the whiteleg shrimp, Litopenaeus vannamei. Environ Pollut. 2023 Jul 15;329:121646. doi: 10.1016/j.envpol.2023.121646. Epub 2023 Apr 25. PMID: 37105466.

[2] 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.

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[4] Du X, Zhou W, Zhang W, Sun S, Han Y, Tang Y, Shi W, Liu G. Toxicities of three metal oxide nanoparticles to a marine microalga: Impacts on the motility and potential affecting mechanisms. Environ Pollut. 2021 Dec 1;290:118027. doi: 10.1016/j.envpol.2021.118027. Epub 2021 Aug 21. PMID: 34428706.

[5] Yu Y, Tian D, Han Y, Huang L, Tang Y, Zhang W, Zhou W, Shi W, Yu Y, Liu G. Impacts of microplastics and carbamazepine on the shell formation of thick-shell mussels and the underlying mechanisms of action. Sci Total Environ. 2022 Sep 10;838(Pt 3):156442. doi: 10.1016/j.scitotenv.2022.156442. Epub 2022 Jun 2. PMID: 35660597.

References:

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

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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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