Fructose 1,6-Bisphosphatase (FBP) 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: BC0925

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	Powder×1	2-8℃
Reagent II	Liquid 8 μL×1	-20℃
Reagent III	Powder×2	-20℃
Reagent IV	Liquid 25 mL×1	2-8℃

Solution Preparation:

1. Reagent I: Dissolve with 20 mL of Reagent IV before use. Unused reagent can separate into small tubules and storage at -20℃ for 4 weeks, avoid repeated freezing and thawing.
2. Reagent II: The liquid is placed in an EP tube inside the bottle. Dissolve with 1.1 mL of distilled water before use. Unused reagent can separate into small tubules and storage at -20℃ for 4 weeks, avoid repeated freezing and thawing.
3. Reagent III: Dissolve one Reagent III with 0.77 mL of distilled water before use. Unused reagent can separate into small tubules and storage at -20℃ for 4 weeks, avoid repeated freezing and thawing. (The reagent is a freeze-dried reagent, there may be a large difference in the amount of reagents observed by the naked eye or even a small amount of the phenomenon, this phenomenon does not affect the use.)

Product Description:

Fructose 1,6-bisphosphatase (FBP) also known as fructose-1,6-diphosphatase, which plays a key role in the gluconeogenesis and the synthesis of photosynthetic assimilate sucrose.

FBP catalyzes fructose 1,6-diphosphate and water to produce 6-phosphate fructose and inorganic phosphorus. Glucose-phosphate isomerase and 6-glucose-phosphate dehydrogenase added to the reaction system that catalyze the formation of 6-glucose-phosphate gluconic acid and NADPH in turn. In this kit, the activity of FBP is determined by the increase rate of NADPH at 340 nm.

Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer/microplate reader, desk centrifuge, adjustable pipette, water bath, micro quartz cuvette/96 well flat-bottom UV plate, mortar/homogenizer/ cell ultrasonic crusher, ice and distilled water.

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Procedure:

I. Sample 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, fully homogenized on ice bath. Centrifuge at 8000 ×g for 10minutes at 4℃ to remove insoluble materials, take the supernatant on ice before testing.

2) Bacteria or cells

According to the Bacteria or cells (10^4): the volume of the Extract solution (mL) is 500~1000:1. It is suggested that add 1mL of Extract solution to 5 million of bacteria or cells. Use ultrasonic to splitting bacteria and cells (placed on ice, ultrasonic power 200W, working time 3 seconds, interval 7 seconds, total time for 3 min). Centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.

II. Determination procedure:

1. Preheat ultraviolet spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 340 nm, set spectrophotometer zero with distilled water.
2. Preheat Reagent I at 37℃(mammal) or 25℃ (other species) for 10 minutes
3. Add the following reagents:

Reagent (μL)	Test tube(T)	Blank tube(B)
Sample	20	-
Extract solution	-	20
Reagent II	10	10
Reagent III	10	10
Reagent I	160	160

Add the above reagents to the micro quartz cuvette/96 well flat-bottom UV plate in order. Detect the absorbance at 340 nm at the time of 10 seconds record as A1. After colorimetry, quickly place the micro quartz cuvette together with the reaction solution into a 37℃(mammal) or 25℃ (other species) water bath or constant temperature incubator to accurately react for 5 minutes. Take it out and wipe it clean, immediately measure the absorbance of final reaction which record as A2 (310s). $\Delta A_T = A_{T2} - A_{T1}$, $\Delta A_B = A_{B2} - A_{B1}$, $\Delta A = \Delta A_T - \Delta A_B$. The blank tube only needs to be tested one or two times.

If the number of samples is too large, reagent II, reagent III and reagent I (after preheating) can be prepared into working solution according to the sample number and the proportion of sample addition in the operation table.

III. Calculation:

A. micro quartz cuvette

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the produce of 1 nmol of NADPH per minute every milligram of protein.

FBP activity (U/mg prot)=[ΔA÷(ε×d)×10^9×Vrv]÷(Vs×Cpr)÷T×F=321.5×ΔA÷Cpr×F

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the produce of 1 nmol of NADPH per minute every gram of tissue.

FBP activity (U/g weight)=[ΔA÷(ε×d)×10^9×Vrv]÷(W÷Ve×Vs)÷T×F =321.5×ΔA÷W×F

3. Bacteria or cultured cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the produce of 1 nmol of NADPH per minute every 1 0000 cells or bacteria.

FBP activity (U/10^4 cell)=[ΔA÷(ε×d)×10^9×Vrv]÷(Vs÷Ve×N)÷T×F =321.5×ΔA÷N×F

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

d: Light path of cuvette, 1 cm;

Vrv: Total reaction volume, 2×10^{-4} L;

Vs: Sample volume, 0.02 mL;

Ve: Extract solution volume, 1 mL;

Cpr: Sample protein concentration (mg/mL);

T: Reaction time, 5 minutes;

W: Sample weight(g);

N: Numbers of cells or bacteria, count by 10^4;

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

F: Dilution factor.

B. 96 well UV plate

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

Note:

1. If ΔA>0.6, please dilute the sample to appropriate concentration, multiply dilute times in the formula.
2. The blank tube is a detection hole for detecting the quality of each reagent component, and normally that the change of ΔA_B does not exceed 0.02.

Experimental example:

1. Take 0.1g of pancreas and add 1mL of extract to the ice bath homogenate. After the supernatant is taken out, the operation is performed according to the determination steps. measure using a micro quartz colorimetric plate, the ΔA_T= A_{T2}-A_{T1}= 0.1957-0.1655=0.0302, ΔA_B = A_{B2}- A_{B1}=

0.0779-0.065= 0.0129, ΔA = Δ A_T- $ΔA_B$ = 0.0302-0.0129=0.0173. And FBP activity is calculated according to the sample mass:

FBP activity (U/g mass) = 321.5×ΔA ÷ W = 321.5×0.0173÷0.1 = 55.6195 U/g mass.

2. Take 0.1g of Ryegrass and add 1mL of extract to the ice bath homogenate. After the supernatant is taken out, the operation is performed according to the determination steps. measure using a micro quartz colorimetric plate, the $ΔA_T$= A_{T2}-A_{T1}= 0.7224-0.6267=0.0957, $ΔA_B$ = A_{B2}- A_{B1}= 0.0779-0.065= 0.0129, ΔA = Δ A_T- $ΔA_B$ = 0.0957-0.0129=0.0828. And FBP activity is calculated according to the sample mass.

FBP activity（U/g mass）=321.5×ΔA÷W=321.5×0.0828÷0.1=266.202 U/g mass.

Recent Product Citation：

[1] Bai D, Du J, Bu X, Cao W, Sun T, Zhao J, Zhao Y, Lu N. ALDOA maintains NLRP3 inflammasome activation by controlling AMPK activation. Autophagy. 2022 Jul;18(7):1673-1693. doi: 10.1080/15548627.2021.1997051. Epub 2021 Nov 25. PMID: 34821530; PMCID: PMC9298449.

[2] Yuan Y, Zhu C, Wang Y, Sun J, Feng J, Ma Z, Li P, Peng W, Yin C, Xu G, Xu P, Jiang Y, Jiang Q, Shu G. Ketoglutaric acid ameliorates hyperglycemia in diabetes by inhibiting hepatic gluconeogenesis via serpina1e signaling. Sci Adv. 2022 May 6;8(18):eabn2879. doi: 10.1126/sciadv.abn2879. Epub 2022 May 4. PMID: 35507647; PMCID: PMC9067931.

[3] Peng L, Lou W, Xu Y, Yu S, Liang C, Alloul A, Song K, Vlaeminck SE. Regulating light, oxygen and volatile fatty acids to boost the productivity of purple bacteria biomass, protein and co-enzyme Q10. Sci Total Environ. 2022 May 20;822:153489. doi: 10.1016/j.scitotenv.2022.153489. Epub 2022 Feb 2. PMID: 35122839.

[4] Liang X, Liu X, Li W, Zhang L, Zhang B, Lai G, Zhao Y. A novel variant in the FBP1 gene causes fructose-1,6-bisphosphatase deficiency through increased ubiquitination. Arch Biochem Biophys. 2023 Jul 1;742:109619. doi: 10.1016/j.abb.2023.109619. Epub 2023 May 2. PMID: 37142076.

[5] Zhu M, He Y, Zhu M, Ahmad A, Xu S, He Z, Jiang S, Huang J, Li Z, Liu S, Hou X, Zhang Z. ipa1 improves rice drought tolerance at seedling stage mainly through activating abscisic acid pathway. Plant Cell Rep. 2022 Jan;41(1):221-232. doi: 10.1007/s00299-021-02804-3. Epub 2021 Oct 25. PMID: 34694441.

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