

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

Cat No: BC0920

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°C
Reagent I	Powder×1	2-8°C
Reagent II	Liquid 22 μL×1	-20°C
Reagent III	Powder×4	-20°C
Reagent IV	Liquid 50 mL×1	2-8°C

Solution Preparation:

1. Reagent I: Dissolve with 45 mL of Reagent IV before use. Unused reagent can separate into small tubules and storage at -20°C for 4 weeks, avoid repeated freezing and thawing.
2. Reagent II: The liquid is placed in an EP tube inside the bottle. Dissolve with 3.05 mL of distilled water before use. Unused reagent can separate into small tubules and storage at -20°C 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°C 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, balance, desk centrifuge, adjustable pipette, water bath, 1 mL quartz cuvette, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

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°C to remove insoluble materials, take the supernatant on ice before testing.

2) Bacteria or cells

According to the Bacteria or cells (10⁴): 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 300W, working time 3 seconds, interval 7 seconds, total time for 3 min). Centrifuge at 8000 ×g for 10 minutes at 4°C to remove insoluble materials and take the supernatant on ice before testing.

II. Determination procedure:

1. Preheat spectrophotometer for 30 minutes, adjust the wavelength to 340 nm, set zero with distilled water.
2. Preheat Reagent I at 37°C(mammal) or 25°C (other species) for 10 minutes
3. Add the following reagents in 1 mL quartz cuvette:

Reagent (μL)	Test tube(T)	Blank tube(B)
Sample	100	-
Extract solution	-	100
Reagent II	50	50
Reagent III	50	50
Reagent I	800	800

Add the above reagents to the 1 mL quartz cuvette in order. Detect the absorbance at 340 nm at the time of 10 seconds record as A1. After colorimetry, quickly place the 1mL quartz cuvette together with the reaction solution into a 37°C(mammal) or 25°C (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:

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.

$$\text{FBP activity (U/mg prot)} = [\Delta A \div (\epsilon \times d) \times 10^9 \times V_{rv}] \div (V_s \times C_{pr}) \div T \times F = 321.5 \times \Delta A \div C_{pr} \times F$$

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.

$$\text{FBP activity (U/g weight)} = [\Delta A \div (\epsilon \times d) \times 10^9 \times V_{rv}] \div (W \div V_e \times V_s) \div T \times F = 321.5 \times \Delta A \div W \times F$$

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.

$$\text{FBP activity (U/10}^4 \text{ cell)} = [\Delta A \div (\epsilon \times d) \times 10^9 \times V_{rv}] \div (V_s \div V_e \times N) \div T \times F = 321.5 \times \Delta A \div N \times F$$

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

d : Light path of cuvette, 1 cm;

V_{rv} : Total reaction volume, 1×10^{-3} L;

V_s : Sample volume, 0.1 mL;

V_e : Extract volume, 1 mL;

C_{pr} : 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, $1 \text{ mol} = 10^9 \text{ nmol}$;

F : Dilution factor.

Note:

1. If $\Delta A > 0.6$, 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 liver 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 1mL quartz colorimetric plate, the $\Delta A_T = A_{T2} - A_{T1} = 0.756 - 0.647 = 0.109$, $\Delta A_B = A_{B2} - A_{B1} = 0.074 - 0.062 = 0.012$, $\Delta A = \Delta A_T - \Delta A_B = 0.109 - 0.012 = 0.097$. And FBP activity is calculated according to the sample mass:

$$\text{FBP activity (U/g mass)} = 321.5 \times \Delta A \div W = 321.5 \times 0.097 \div 0.1 = 311.855 \text{ U/g mass.}$$

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 1mL quartz colorimetric plate, the $\Delta A_T = A_{T2} - A_{T1} = 0.785 - 0.609 = 0.176$, $\Delta A_B = A_{B2} - A_{B1} = 0.074 - 0.062 = 0.012$, $\Delta A = \Delta A_T - \Delta A_B = 0.176 - 0.012 = 0.164$.

$A_{BI}=0.074-0.062=0.012$, $\Delta A = \Delta A_T - \Delta A_B = 0.176-0.012=0.164$. And FBP activity is calculated according to the sample mass:

FBP activity (U/g mass) = $321.5 \times \Delta A \div W = 321.5 \times 0.164 \div 0.1 = 527.26$ U/g mass.

Recent Product Citation:

[1] Zhou WJ, Yang HL, Mei J, Chang KK, Lu H, Lai ZZ, Shi JW, Wang XH, Wu K, Zhang T, Wang J, Sun JS, Ye JF, Li DJ, Zhao JY, Jin LP, Li MQ. Fructose-1,6-bisphosphate prevents pregnancy loss by inducing decidual COX-2+ macrophage differentiation. *Sci Adv.* 2022 Feb 25;8(8):eabj2488. doi: 10.1126/sciadv.abj2488. Epub 2022 Feb 23. PMID: 35196096; PMCID: PMC8865779.

[2] Ren YR, Ye YL, Feng Y, Xu TF, Shen Y, Liu J, Huang SL, Shen JH, Leng Y. SL010110, a lead compound, inhibits gluconeogenesis via SIRT2-p300-mediated PEPCK1 degradation and improves glucose homeostasis in diabetic mice. *Acta Pharmacol Sin.* 2021 Nov;42(11):1834-1846. doi: 10.1038/s41401-020-00609-w. Epub 2021 Feb 11. PMID: 33574568; PMCID: PMC8563938.

[3] 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 serpinale signaling. *Sci Adv.* 2022 May 6;8(18):eabn2879. doi: 10.1126/sciadv.abn2879. Epub 2022 May 4. PMID: 35507647; PMCID: PMC9067931.

[4] 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.

[5] 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.

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