# Fructose 1,6-Bisphosphatase (FBP) Activity Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer

**Cat No:** NA0801

**Size:**50T/48S

**Components:**

Extract solution: Liquid 60 mL×1. Store at 4℃.

Reagent I: Powder×1. Store at -20℃. Dissolve with 45 mL of Reagent IV before use. Unused reagent store at 4℃.

Reagent II: Liquid 18 μL×1. Store at -20℃. Dissolve with 2.5 mL of distilled water before use. Unused reagent can separate into small tubules and storage at -20℃, avoid repeated freezing and thawing.

Reagent III: Liquid 245 μL×1. Store at -20℃. Dissolve with 2.5 mL of distilled water before use. Unused reagent can separate into small tubules and storage at -20℃, avoid repeated freezing and thawing.

Reagent IV: Liquid 50 mL×1. Store at 4℃.

**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, desk centrifuge, adjustable pipette, water bath, 1 mL quartz cuvette, mortar/homogenizes, 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. Suggest 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. Suggest 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℃ 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℃(mammal) or 25℃(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, timing after add working solution, mix thoroughly. Detect the absorbance at 340 nm at the time of 10 seconds record as $A_{T1}$ or $A_{B1.}$ Then place dishes with the reaction solution in a 30℃ water bath for 5 minutes. Take it out and wipe it clean, immediately measure the absorbance at the time of 310 seconds which record as $A_{T2}$ or $A_{B2}$. $\Delta A_T = A_{T1}-A_{T2}$, $\Delta A_B = A_{B1}- A_{B2}$, $\Delta A=\Delta A_T-\Delta A_B$. The blank tube only need to be tested one or two times. | | |

**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.

$FBP(U/mg\ prot)=[\Delta A\div(\varepsilon\times d)\times10^9\times Vrv]\div(Vs\times Cpr)\div T=321.5\times\Delta A\div Cpr$

**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.

$FBP(U/g\ weight)=[\Delta A\div(\varepsilon\times d)\times10^9\times Vrv]\div(W\div Ve\times Vs)\div T=321.5\times\Delta A\div W$

**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.

$FBP(U/10^4\ cell)=[\Delta A\div(\varepsilon\times d)\times10^9\times Vrv]\div(Vs\div Ve\times N)\div T=321.5\times\Delta A\div N$

ε: NADPH molar extinction coefficient, $6.22\times10^3$ L/mol/cm;

d: Light path of cuvette, 1 cm;

Vrv: Total reaction volume,$1\times10^{-3}$ L;

Vs: Sample volume, 0.1 mL;

Ve: Extract volume, 1 mL;

Cpr: Sample protein concentration (mg/mL);

T: Reaction time, 5 minutes;
W: Sample weight(g);
N: Numbers of cells or bacteria (unit: $10^4$);
$10^9$: 1 mol =$10^9$ nmol.

**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 $\Delta A_B$ does not exceed 0.02.

**Experimental example:**

1. 1 mL of Extract solution is added to 0.1 g of liver for homogenization. After the supernatant is taken out, the operation is performed according to the determination steps. measure using a micro quartz colorimetric plate, the $\Delta A_T$= $A2_T$-$A1_T$= 0.756-0.647=0.109, $\Delta A_B$ = $A2_B$-$A1_B$= 0.074-0.062=0.012, ΔA = Δ $A_T$- $\Delta A_B$ = 0.109-0.012=0.097.

FBP (U/g mass) = 321.5×ΔA÷W=321.5×0.097÷0.1=311.855 U/g mass.

2. 1 mL of Extract solution is added to 0.1 g of Ryegrass for homogenization. After the supernatant is taken out, the operation is performed according to the determination steps. measure using a micro quartz colorimetric plate, the $\Delta A_T$= $A2_T$-$A1_T$=0.785-0.609=0.176, $\Delta A_B$ = $A2_B$-$A1_B$=0.074-0.062=0.012, ΔA = Δ $A_T$- $\Delta A_B$ =0.176-0.012=0.164.

FBP（U/g mass）=321.5×ΔA÷W=321.5×0.164÷0.1=527.26 U/g mass

**Related Products**：

NA0810/NA0568 Pyruvate Carboxylase(PC) Activity Assay Kit
NA0635/NA0393 Phosphoenolpyruvate Carboxykinase(PEPCK) Activity Assay Kit
NA0634/NA0392 Glucose-6-phosphatase Activity Assay Kit