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

Note: Take two or three different samples for prediction before test.
Operation Equipment: Spectrophotometer/Microplate Reader

Cat No: AK0275
Size:100T/96S

Components:

Extract solution: Liquid 100 mL×1. Store at 4℃ .

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

Reagent II: Liquid 8 μL×1. Store at -20℃ . Dissolve with 1.1 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 108 μL×1. Store at -20℃ . Dissolve with 1.1 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 25 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/ Microplate Reader, desk centrifuge, adjustable pipette, water bath, micro quartz cuvette/96 well flat-bottom UV plate, mortar/homogenizer, 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:

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, 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:

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.

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

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, 6.22×10^3 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 (unit: 10^4);
10^9: 1 mol =10^9 nmol.

B. 96 well UV plate

Change the d- 1cm in the above formula to d-0.6cm (the optical diameter of cuvette) 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. 1 mL of Extract solution is added to 0.1 g of pancreatic tissue 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 ΔA_T= $A2_T$-$A1_T$= 0. 1957-0. 1655 = 0.0302, ΔA_B = $A2_B$-$A1_B$= 0.0779-0.065 = 0.0129, ΔA = Δ A_T- ΔA_B = 0.0302-0.0129 = 0.0173.

FBP (U/g mass) = 321.5×ΔA ÷ W = 321.5×0.0173÷0. 1 = 55.6195 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 ΔA_T= $A2_T$-$A1_T$=0.7224-0.6267=0.0957, ΔAB = A2B-A1B=0.0779-0.065=0.0129, ΔA = Δ A_T- ΔA_B =0.0957-0.0129=0.0828.

FBP（U/g mass）=321.5×ΔA÷W=321.5×0.0828÷0. 1=266.202 U/g mass

Related Products：

AK0317/AK0316	Pyruvate Carboxylase(PC) Activity Assay Kit
AK0143/AK0142	Phosphoenolpyruvate Carboxykinase(PEPCK) Activity Assay Kit
AK0141/AK0140	Glucose-6-phosphatase Activity Assay Kit