Fructose-1,6-bisphosphate aldolase (FBA) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: BC2275

Size:100T/96S

Components:

Extract solution I: Liquid 110 mL×1. Store at 4℃.

Extract solution II: Liquid 110 mL×1. Store at 4℃.

Reagent I: 10 mL×1. Store at 4℃.

Reagent II: Powder×1. Store at -20℃. Dissolve with 2 mL of distilled water before use. Unused reagent can separate into small tubules and storage at -20℃, avoid repeated freezing and thawing.

Reagent III: Powder×1. Store at 4℃. Dissolve with 2 mL of distilled water before use. Unused reagent can store at 4℃.

Reagent IV: Liquid×1. Store at 4℃. Dissolve with 2 mL of distilled water before use. Unused reagent can separate into small tubules and storage at -20℃, avoid repeated freezing and thawing.

Reagent V: Liquid×1. Store at 4℃. Dissolve with 2 mL of distilled water before use. Unused reagent can separate into small tubules and storage at -20℃, avoid repeated freezing and thawing.

Product Description:

Fructose 1,6 bisphosphate aldolase (FBA) (EC4.1.2.13) is an important part of the Calvin cycle in glycolysis, gluconeogenesis, pentose phosphate pathway, and photosynthesis Enzymes, which catalyze the reversible cleavage of fructose 1,6-diphosphate to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, are widely present in plants, animals and microorganisms, and exhibit different responses under various stress conditions.

FBA catalyzes the production of fructose 1,6-diphosphate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, and catalyzes NADH and triphosphate under the action of triose phosphate isomerase and α-phosphate glycerol dehydrogenase. Dihydroxyacetone phosphate produces NAD and α-phosphoglycerin. The change in absorbance at 340nm can reflect the level of fructose 1,6-bisphosphate aldolase activity.

Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer/microplate reader, desk centrifuge, adjustable transferpettor, micro quartz cuvette/ 96 well UV plate, mortar/homogenizer, vortex oscillator, ultrasonic crusher, ice and distilled water.

Procedure:

I. Extraction of crude enzyme solution:

A. Total FBA 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 I to 0.1 g of tissue, fully homogenized on ice bath. Centrifuge at 8000 ×g for 10 minutes 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 1 mL of Extract solution I to 5 million of bacteria or cells. Use ultrasonication 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.

3) Liquid: detect directly

B. FBA preparation in cytoplasm and chloroplast:

(1) According to the quality of plant tissue (g): the volume of the Extract solution (mL) is 1: 5-10 (recommended to weigh about 0.1 g sample and add 1 mL Extract solution I), quickly grind or homogenize, and then Centrifuge at 200 g for 5 min at 4℃;

(2) Discard the pellet and take the supernatant at 4℃ and centrifuge at 8000 g for 10 min (slow acceleration and deceleration during centrifugation);

(3) **Take the supernatant to measure the cytosolic FBA enzyme activity, take the precipitate and add 1 mL of Extract solution II,** and then sonicate it after dissolution by shaking (ice bath, 200W, crushing 3s, intermittent 7s, total time 1 min), then 4℃, 8000 g centrifugation for 10 min, **the supernatant is the FBA enzyme activity in the chloroplast.**

It is recommended to measure the total FBA enzyme activity and extract the crude enzyme solution according to step I. If the FBA in the cytoplasm and chloroplast are to be measured separately, then extract the crude enzyme solution according to step II.

II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 340 nm, set zero with distilled water.
2. Add the following reagents in 1 mL quartz cuvette:

Reagent name (μL)	Test tube (T)	Blank tube (B)
Reagent I	100	100
Reagent II	20	20
Reagent III	20	20
Reagent IV	20	20
Reagent V	20	20
Sample	20	
Distilled water		20

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 37℃ (mammal) or 25℃(other species) water bath for 5 minutes (Microplate reader with temperature control function can adjust the temperature to 37℃ or 25℃). 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.

Note: If the amount of test sample is large, Reagent I, II, III, IV, V can be prepared into a working solution at a ratio of 5: 1: 1: 1: 1 (V: V: V: V: V) for use.

III. Calculation:

1. Micro quartz cuvette:

(1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 nmol of NADH in the reaction system per minute every mg protein.

FBP (U/mg prot) $=[\Delta A \div (\varepsilon \times d) \times 10^9 \times Vrv] \div (Vs \times Cpr) \div T = 321.5 \times \Delta A \div Cpr$

(2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 nmol of NADH in the reaction system per minute every g sample.

FBP (U/g weight) $=[\Delta A \div (\varepsilon \times d) \times 10^9 \times Vrv] \div (W \div Ve \times Vs) \div T = 321.5 \times \Delta A \div W$

(3) Bacteria or cultured cells

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 nmol of NADH in the reaction system per minute every 10^4 Bacteria or cells.

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

(4) Liquid volume

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 nmol of NADH in the reaction system per minute every mL liquid.

FBP (U/mL) $=[\Delta A \div (\varepsilon \times d) \times 10^9 \times Vrv] \div Vs \div T = 321.5 \times \Delta A$

Vrv: Total reaction volume, 0.001 L;

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

d: Light path of cuvette, 1 cm;

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.

2. 96 well flat-bottom plate:

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

Note:

1. If ΔA>0.8, please dilute the sample to appropriate concentration, multiply dilute times in the formula.
2. If it is a **plant sample**, it is recommended to test it within 2 hours after the extraction is completed. If the sample size is too large, it is recommended to extract and test it in batches.
3. Because the extract solution contains a certain concentration of protein (about 0.5 mg/mL), it is necessary to subtract the protein content of the extract solution itself when determining the protein concentration of the sample.

Experimental example:

1. Take 0.1g mouse liver and add 1ml Extract solution for homogenization, take the supernatant and dilute 8 times, then operate according to the determination steps. Measure using micro quartz cuvette and calculate $\Delta A_T = A1_T - A2_T = 1.3061-1.125 = 0.1811$, $\Delta A_B = A1_B - A2_B = 1.1983-1.1858 = 0.0125$, $\Delta A = \Delta A_T - \Delta A_B = 0.1811-0.0125 = 0.1686$

FBA activity (U/g mass) = 321.54 × ΔA ÷ W × 8 (dilution) = 321.54 × 0.1686 ÷ 0.1 × 8 (dilution) = 4337 U/g mass.

2. Take 0.1g of Mallotus aureus and add 1 mL of Extract solution for homogenization. After taking the supernatant, operate according to the determination steps. Measure using micro quartz cuvette and calculate $\Delta A_T = A1_T - A2_T = 1.4458-1.37 = 0.0758$, $\Delta A_B = A1_B - A2_B = 1.1983-1.1858 = 0.0125$, $\Delta A = \Delta A_T - \Delta A_B = 0.0758-0.0125 = 0.0633$.

FBA activity (U/g mass) = 321.54×ΔA ÷ W = 321.54×0.0633 ÷ 0.1 = 204 U/g mass.

Related Products：

BC2240/BC2245 Fructose-1，6-diphosphate(FDP) Assay Kit

BC0530/BC0535 Phosphofructokinase(PFK) Activity Assay Kit