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Fructose 1,6-bisphosphatase (FBP) Assay Kit (Microplate method)

Product Number: BC2273

Shipping and Storage

1. Reagent 1 and Reagent 2 : Store at -20℃;
2. Reagent 3: Store at 4℃ away from light;
3. Extraction Solution, Standard, Reagent 1、4 and Reagent 5: Store at 4℃.
4. Validity period: 3 months.

Component

Component	96T
Extraction Solution	100mL× 1 bottle
Reagent 1	Powder, 1 vial
Reagent 2	Powder, 1 vial
Reagent 3	1mL × 1 bottle
Reagent 4	20mL × 1 bottle
Reagent 5	Powder × 1 bottle
Standard	Powder, 1 vials

1. Reagent 1: Before use, centrifuge at 8000 g, 4℃ for 2 min to collect reagent at the tube bottom (manual shaking is acceptable). Add 1.1mL of distilled water for reconstitution prior to use.Valid for the same shelf-life as the kit.
2. Reagent 2: Before use, centrifuge at 8000 g, 4℃ for 2 min to pellet powder to the tube bottom (manual shaking is acceptable). Add 1.1mL distilled water to dissolve each vial. Aliquot unused portions and store at −20℃.
3. Reagent 5: Ensure the powder falls to the bottom before opening the vial (gentle manual shaking is allowed); add 2.1mL distilled water to dissolve for later use. Its shelf life is consistent with the expiry date of the kit.
4. Standard: This reagent is required if the standard curve is re-prepared. Prepare the standard according to the standard curve preparation procedure described in the manual. The dissolved standard should be used up within one week.

Description

Fructose-1,6-bisphosphatase, also known as fructose 1,6-diphosphatase (FBP, EC 3.1.3.11), is a key enzyme in the gluconeogenesis pathway. Different gluconeogenic substrates are converted into fructose 1,6-bisphosphate under the action of multiple enzymes, which is then hydrolyzed to fructose 6-phosphate and inorganic phosphate catalyzed by FBP. Abnormal expression of this enzyme is closely associated with certain diseases.

This kit provides a simple, sensitive and rapid assay method: FBP catalyzes the reaction of fructose 1,6-bisphosphate with water to produce fructose 6-phosphate and inorganic phosphate, which interacts with the enzymatic complex. The NADPH generated in this process subsequently reacts with a specific chromogenic probe to form colored substances. The activity of FBP is calculated by measuring the rate of increase of the colored substances.

Required Laboratory Equipment

Mortar (homogenizer), ice box (ice-maker), table-top centrifuge, adjustable pipettors, water bath (oven / incubator / metal heat block), 1mL quartz cuvettes, centrifuge tubes, UV-visible spectrophotometer, distilled water (deionized water or ultrapure water is acceptable).

Sample Assay

It is recommended to run a pilot test with 1-3 representative samples of distinct groups/types to get familiar with the workflow. Adjust sample loading concentration according to pilot results to avoid unnecessary consumption of samples and reagents.

1. **Sample Extraction**

1.1. Tissue Samples

Weigh 0.1g tissue sample, add 1 mL Extraction Solution, and perform ice-bath homogenization. Centrifuge homogenate at 12 000 rpm, 4℃ for 10 min. Collect supernatant for subsequent measurement.

Note: For larger sample input, perform extraction at a mass-to-volume ratio: tissue weight (g) : Extraction Solution volume (mL) = 1 : 5 ~ 10.

1.2. Bacterial / Cell Samples

Collect bacteria or cells into a centrifuge tube, discard the supernatant after centrifugation. Add 1mL of extraction solution to 5×10^6 bacteria or cells, and homogenize at 4°C or in an ice bath (common electric homogenizers can be used). Centrifuge at approximately 12,000 rpm for 10 min at 4°C, then collect the supernatant for assay.

Note: For larger sample input, perform extraction at ratio: cell count ($\times10^4$ cells) : Extraction Solution (mL) = 500~ 1000 :1. Cell lysis using commercial lysis buffer is also acceptable; follow the lysis-buffer manufacturer's instructions.

2. **Protocol**

2.1. Pre-warm microplate reader for 30 min. Set wavelength to 340 nm and temperature to 25°C.

2.2. Thaw the reagents to room temperature (25°C) or incubate in a water bath (25°C) for 15–30 min.

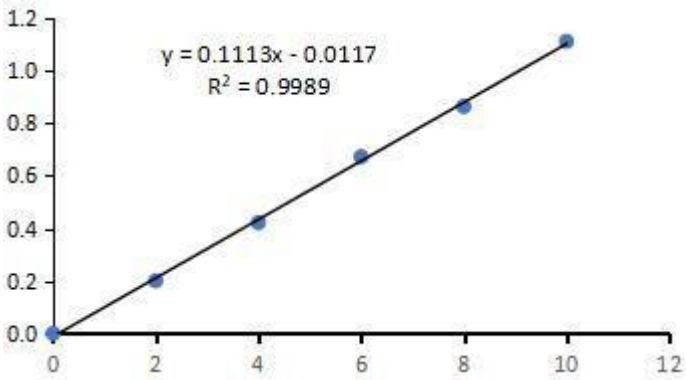
2.3. Pipette reagents sequentially into wells of a 96-well microplate:

Component	Assay tube
Sample	10μL
Reagent 1	10μL
Reagent2	10μL
Reagent 3	10μL
Reagent 4	140μL
Mix gently and incubate at room temperature (25°C) for 5 min.	
Reagent 5	20μL
Mix gently. Record absorbance A1 at 1 min and absorbance A_2 at 5 min at 340nm under 25 ℃. Calculate ΔA = A1 − A2.	

Note: If ΔA is less than 0.01, extend the reaction time T (e.g., re-read A2 after increasing to 25 min), or increase V1 (e.g., increase to 50μL with a corresponding reduction in Reagent 4). The modified T and V1 must be re-substituted into the formula for calculation.

Calculation of Results

1. Standard curve equation: y = 0.1113x − 0.0117, where x is the molar mass of the standard (nmol) and y is △A.



2. Calculation based on sample protein concentration:

Enzyme-activity definition: One unit of enzyme activity is defined as consumption of 1nmol NADH per minute per milligram of tissue protein.

FBP(nmol/min/mg prot)=[(ΔA+0.0117)÷0.1113]÷(V1×Cpr)÷T=59.9×(ΔA+0.0117)÷Cpr

3. Calculation based on sample fresh weight

Enzyme-activity definition: One unit of enzyme activity is defined as consumption of 1nmol NADPH per minute per gram fresh-weight tissue.

FBP(nmol/min/g fresh weight)=[(ΔA+0.0117)÷0.1113]÷(W×V1÷V)÷T=59.9×(ΔA+0.0117)÷W

4. Calculation based on cell number

Enzyme-activity definition: One unit of enzyme activity is defined as consumption of 1 nmol NADPH per minute per 1×10^4 cells.

FBP(nmol/min/10^4 cell)=[(ΔA+0.0117)÷0.1113]÷(W×V1÷V)÷T=59.9×(ΔA+0.0117)÷500.

Parameter explanations:

V_1 — Volume of sample loaded in reaction = 0.01mL

T — Reaction incubation time = 15 min

W — Sample fresh weight (g)

500 — Cell count unit: ten-thousand cells

Cpr — Sample protein concentration (mg/mL). BCA Protein Quantification Kit from our company is recommended for protein determination.

Appendix: Preparation Procedure of Standard Curve

1. Add 0.6mL distilled water to the EP tube containing the standard (the stock solution must be used within two days and stored at -20°C). The concentration of the standard stock solution is 1nmol/μL. Dilute the stock solution with distilled water to prepare standards at six concentration gradients, for example: 0, 0.2, 0.4, 0.6, 0.8, 1nmol/μL. The standard concentrations may also be adjusted according to actual samples.
2. See the table below for standard dilution reference:

Standard Concentration (nmol/μL)	0	0.2	0.4	0.6	0.8	1
Standard Diluent μL	0	40	80	120	160	200
H_2O μL	200	160	120	80	40	0
Mix each standard tube well and set aside for use.						

3. Operate according to the pipetting table. Subtract the absorbance of the 0-concentration standard from the absorbance of each concentration based on the results, then plot the standard curve passing through the origin.

Reagent Name (μL)	Standard Tube	0-Concentration Tube (prepare only once)
Standard	10	
Distilled Water		10
Reagent 3	10	10
Distilled Water	40	40
Reagent 4	140	140
Mix well and read absorbance A at 450nm. ΔA = A_sample − 0-concentration tube.		