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

Cat #: KTB1331 Size: 48 T/96 T

	Micro Fructose 1,6-Bisphosphatase (FBP) Activity Assay Kit		
REF	**Cat #:** KTB1331	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant tissues, Cells		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Fructose -1,6- diphosphatase (FBP), also known as fructose -1,6- diphosphatase, plays a key role in gluconeogenesis and the synthesis of sucrose, an assimilate of photosynthesis. Catalyze fructose-1,6- diphosphate and water to produce fructose-6-phosphate and inorganic phosphorus. CheKine™ Micro Fructose 1,6-Bisphosphatase (FBP) Activity Assay Kit can detect animal and plant tissues, cells, plasma, serum or other liquid samples. In this kit, FBP catalyzes fructose-1,6-diphosphate and water to produce fructose-6-phosphate and inorganic phosphorus. Glucose-6-phosphate isomerase and glucose-6-phosphate dehydrogenase added in the reaction system catalyze glucose-6-phosphate and NADPH in turn, and the activity of FBP can be calculated by measuring the increase rate of NADPH at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	1	1	-20℃, protected from light
Reagent Ⅱ	3.6 μL	7.2 μL	4℃, protected from light
Reagent Ⅲ	1	1	-20℃, protected from light
Reagent Ⅳ	12.5 mL	25 mL	4℃

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV microplate or micro quartz cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, cryogenic centrifuge
- Deionized water
- Mortar or homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent I: Prepared before use. Add 10 mL Reagent Ⅳ for 48 T and 20 mL Reagent Ⅳ for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

Reagent II: Prepared before use. Add 0.5 mL deionized water for 48 T and 1 mL deionized water for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

Reagent III: Prepared before use. Add 0.5 mL deionized water for 48 T and 1 mL deionized water for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

Reagent Ⅳ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Reagent Ⅰ、Ⅱ、Ⅲ place at 37°C (mammal) or 25°C (other species) incubation for 10 min.
3. Operation table (The following operations are operated in the 96-well UV microplate or micro quartz cuvette **in turn**):

Reagent	Blank Well (μL)	Test Well (μL)
Sample	0	20
Extraction Buffer	20	0
Reagent Ⅱ	10	10
Reagent Ⅲ	10	10
Reagent Ⅰ	160	160

4. Mix thoroughly, measure the absorbance value A_1 at 10 s at 340 nm, and the absorbance value A_2 at 310 s at 37℃ for 5 min. The Test Well is marked as A_{Test}, the Blank Well is marked as A_{Blank}. Finally calculate $\Delta A=(A_{2Test}-A_{1Test})-(A_{2Blank}-A_{1Blank})$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.05, increase the sample quantity appropriately. If ΔA is greater than 0.6, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately. If ΔA is negative, the sample does not contain FBP or is degraded.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

Calculation of FBP activity:

A. 96-well UV plates calculation formula as below

(1) Calculated by protein concentration

Active unit definition: The production of 1 nmol of NADPH per milligram of protein per min was defined as one unit of enzyme activity.

FBP (U/mg prot)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}×Cpr)÷T**=321.54×ΔA÷Cpr**

(2) Calculated by fresh weight of samples

Active unit definition: The production of 1 nmol of NADPH per gram tissue per min was defined as one unit of enzyme activity.

FBP (U/g fresh weight)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(W×V_{Sample}÷$V_{Total\ sample}$)÷T**=321.54×ΔA÷W**

(3) Calculated by cell number

Active unit definition: The production of 1 nmol of NADPH per 10^4 cell min was defined as one unit of enzyme activity.

FBP (U/10^4 cell) = [ΔA×V_{Total}÷(ε×d)×10^9]÷(n×V_{Sample}÷$V_{Total\ sample}$)÷T**=321.54×ΔA÷n**

V_{Total}: total reaction volume, 2×10^{-4} L; ε: NADPH molar extinction coefficient, 6.22×10^3 L/mol /cm; d: the light path of the 96-well plate, 0.5 cm; V_{Sample}: sample volume added, 0.02 mL; $V_{Total\ sample}$: Extraction Buffer volume added, 1 mL; T: reaction time, 5 min; Cpr: sample protein concentration, mg/mL; W: weight of sample, g; n: Total number of cells, calculated in units of ten thousand.

B. Micro quartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

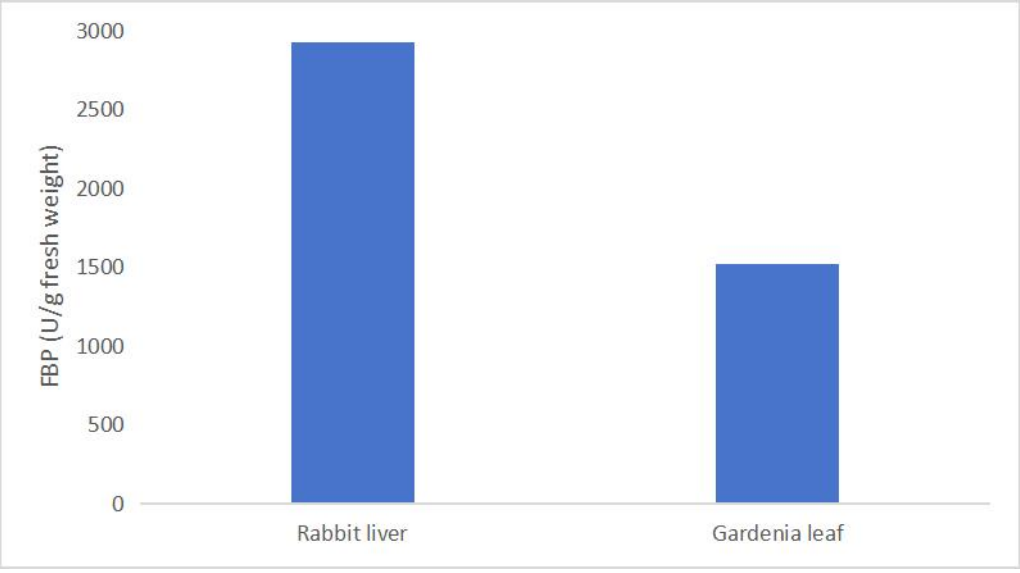

Figure 1. Determination FBP activity in rabbit liver and gardenia leaf by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB3030	CheKine™ Micro Alcohol Dehydrogenase (ADH) Activity Assay Kit

KTB1560	CheKine™ Micro Alcohol Acyltransferase (AAT) Activity Assay Kit
KTB1270	CheKine™ Micro Pyruvate Dehydrogenase (PDH) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.