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CheKine™ Micro Fructose 1,6-Bisphosphate Aldolase (FBA) Activity Assay Kit

Cat #: KTB1332 Size: 48 T/96 T

	Micro Fructose 1,6-Bisphosphate Aldolase (FBA) Activity Assay Kit		
REF	**Cat #:** KTB1332	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant tissues, Cells, Fungus, Plasma, Serum or other Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Fructose 1,6-diphosphate aldolase (FBA)(EC.4.1.2.13) is an important enzyme involved in calvin cycle in glycolysis, gluconeogenesis, pentose phosphate pathway and photosynthesis, which catalyzes the reversible cleavage of fructose 1,6-diphosphate into dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, and is widely used. CheKine™ Micro Fructose 1,6-Bisphosphate Aldolase (FBA) Activity Assay Kit can detect animal and plant tissues, cells, fungus, plasma, serum or other liquid samples. In this kit, FBA catalyzes fructose-1,6-diphosphate to produce glyceraldehyde-3-phosphate and dihydroxyacetone-3-phosphate, and NADH and dihydroxyacetone-3-phosphate are catalyzed to produce NAD and α-glycerophosphate under the action of triose phosphate isomerase and α-glycerophosphate dehydrogenase. The change of absorbance at 340 nm can reflect the activity of FBA.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer Ⅰ	50 mL	100 mL	4℃
Extraction Buffer Ⅱ	50 mL	100 mL	4℃
Reagent Ⅰ	5 mL	10 mL	4℃, protected from light
Reagent Ⅱ	1	1	-20℃, protected from light
Reagent Ⅲ	1	1	4℃, protected from light
Reagent Ⅳ	4 μL	8 μL	-20℃, protected from light
Reagent Ⅴ	40 μL	80 μL	-20℃, protected from light

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

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

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Reagent II: Prepared before use. Add 1 mL deionized water for 48 T and 2 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 1 mL deionized water for 48 T and 2 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: Prepared before use. Add 1 mL deionized water for 48 T and 2 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 V: Prepared before use. Add 0.96 mL deionized water for 48 T and 1.92 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.

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: ① **Extraction of total FBA:** weigh about 0.1 g sample, add 1ml of Extraction Buffer Ⅰ, homogenize in ice bath, and ultrasonically crush for 30 times (power 20% or 200 W, ultrasonic for 3 s, interval 7 s), then centrifuge at 4℃ for 10 min at 8,000 g, and take the supernatant and put it on ice for testing. ② **Separation of plant tissue FBA cytoplasm and chloroplast:** Weigh about 0.1 g sample, add 1 ml of Extraction Buffer Ⅰ, homogenize 200 g in ice bath, centrifuge at 4℃ for 5 min, discard the precipitate, take 8,000 g of supernatant, centrifuge at 4℃ for 10 min, **take the supernatant to determine the activity of cytoplasmic FBA**, and add 1 ml of Extraction Buffer Ⅱ to the precipitate. After shaking and dissolving, it was crushed by ultrasonic for 30 times (power 20% or 200 W, ultrasonic for 3 s, interval 7 s), then 8,000 g was centrifuged at 4℃ for 10 min, **and the supernatant was taken to determine the FBA activity of chloroplasts**.

Note: For plant tissues, it is recommended to determine the total FBA activity and extract the crude enzyme solution according to step ①. If FBA in cytoplasm and chloroplast needs to be determined separately, extract the crude enzyme solution according to step ②.

2. Cells or Fungus: Collect 5×10^6 cells or fungus into the centrifuge tube, wash cells or fungus with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer Ⅰ to ultrasonically disrupt the cells or fungus 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.

3. Serum, Plasma or other Liquid samples: Test directly.

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 place at 37℃ 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)
Reagent Ⅰ	100	100
Reagent Ⅱ	20	20
Reagent Ⅲ	20	20
Reagent Ⅳ	20	20
Reagent Ⅴ	20	20
Deionized Water	20	0
Sample	0	20

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 10 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 0.8, 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 FBA 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 FBA activity:

A. 96-well UV plates calculation formula as below

(1) Calculated by protein concentration

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

FBA (U/mg prot)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div(V_{Sample}\times Cpr)\div T$**$=321.54\times\Delta A\div Cpr$**

(2) Calculated by fresh weight of samples

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

FBA (U/g fresh weight)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div(W\times V_{Sample}\div V_{Total\ sample})\div T$**$=321.54\times\Delta A\div W$**

(3) Calculated by cell or fungus number

Active unit definition: The consumption of 1 nmol of NADH per 10^4 cell or fungus min was defined as one unit of enzyme activity.

FBA ($U/10^4$) = $[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div(n\times V_{Sample}\div V_{Total\ sample})\div T$**$=321.54\times\Delta A\div n$**

(4) Calculated by volume of samples

Active unit definition: The consumption of 1 nmol of NADH per mL liquid per min was defined as one unit of enzyme activity.

FBA (U/mL) = $[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div(V_{Sample}\div V_{Total\ sample})\div T$**$=321.54\times\Delta A$**

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 or fungus, 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.

Precautions

1. If the sample size is large, Reagent Ⅰ, Ⅱ, Ⅲ, Ⅳ and Ⅴ can be made into Working Reagent according to the ratio of 100 μl: 20 μl: 20 μl: 20 μl: 20 μl, and each well needs 180 μL Working Reagent, which is is freshly prepared.
2. If it is a plant sample, it is recommended to complete the detection within 2 h after the extraction is completed. If the sample size is too large, it is recommended to extract and detect it in batches.

Typical Data

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

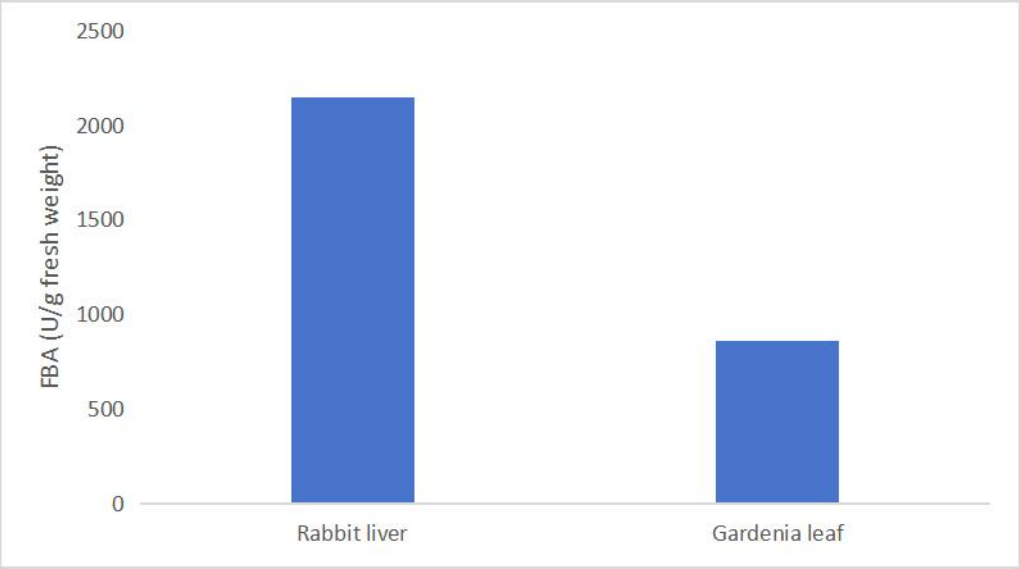

Figure 1. Determination FBA activity in rabbit liver and gardenia leaf by this assay kit.

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.