Fructose-1,6-Biphosphate Aldoalase (FBA) Activity Assay Kit (Spectrophotometer)

Cat. No. KITG-091 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This assay kit is designed for the quantitative determination of fructose-1,6-bisphosphate aldolase (FBA) activity in biological samples using spectrophotometric detection at 340 nm. The assay is based on a coupled enzymatic reaction in which FBA catalyzes the reversible cleavage of fructose-1,6-bisphosphate into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. The subsequent reactions catalyzed by triose phosphate isomerase (TPI) and α-glycerophosphate dehydrogenase cause NADH to be oxidized to NAD^+. The decrease in NADH concentration is monitored at 340 nm and is proportional to FBA activity. Fructose-1,6-bisphosphate aldolase is widely present in animals, plants, and microorganisms. It serves as an important enzyme in glycolysis, gluconeogenesis, the pentose phosphate pathway, and the Calvin cycle of photosynthesis, and exhibits different responses under various stress conditions.
Species	Animals; Plants; Microorganisms
Description	Assay principle: FBA catalyzes the formation of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate from fructose-1,6-bisphosphate. Under the action of triose phosphate isomerase (TPI) and α-glycerophosphate dehydrogenase (α-GPDH), NADH and dihydroxyacetone phosphate are converted to NAD^+ and α-glycerophosphate. NADH has a characteristic absorption peak at 340 nm, and the decrease in absorbance is used to characterize FBA activity. Detection method: Ultraviolet spectrophotometry at 340 nm. Target species: Animals, plants, and

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	microorganisms. Compatible sample types: Animal tissue homogenates, plant tissue homogenates, bacterial cell lysates, mammalian cell lysates, and liquid samples. Size: 50 Tests / 48 Samples. Pathways: Glycolysis / gluconeogenesis; pentose phosphate pathway; Calvin cycle (photosynthesis). Function: Catalyzes the reversible cleavage of fructose-1,6-bisphosphate into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. It plays a central role in carbohydrate metabolism, including glycolysis, gluconeogenesis, and the Calvin cycle, and responds to various environmental stress conditions. Features and benefits: Convenient spectrophotometric detection at 340 nm; suitable for tissues, cells, bacteria, and liquid samples; protocols are provided for total FBA, cytoplasmic FBA, and chloroplastic FBA extraction; comprehensive calculation formulas are provided for different sample types; high-sensitivity NADH-based detection; and reagents are stable when stored under the recommended conditions.
Form	Kit
Applications	Measurement of fructose-1,6-bisphosphate aldolase (FBA) activity in tissue homogenates, cell lysates, and liquid samples. Suitable for research related to glycolysis, gluconeogenesis, the pentose phosphate pathway, and the Calvin cycle, and for investigating stress responses in plants, animals, and microorganisms.
Notes	Important Notes 1. If ΔA is greater than 0.8, dilute the crude enzyme solution with the corresponding extraction buffer before measurement and adjust the calculation accordingly. 2. When measuring plant samples, complete the detection within 2 hours after extraction. If the sample volume is large, extract and detect the samples in batches. 3. Extraction Buffer A contains approximately 0.5 mg/mL protein. When determining the sample protein concentration, subtract the protein content contributed by Extraction Buffer A. 4. Reagents 1, 2, 3, 4, and 5 may be mixed at a volume ratio of 5:1:1:1:1 to prepare a working solution. Prepare the working solution fresh and use it immediately. 5. To ensure accurate results and avoid reagent loss, carefully read

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the instructions before measurement, confirm that reagent storage and preparation are adequate, and make sure that the operating steps are clear. Perform a preliminary determination using 2-3 samples expected to show large differences. Handling 1. Pre-warm the spectrophotometer for more than 30 minutes and set the wavelength to 340 nm. Zero the instrument with distilled water. 2. Pre-warm the required volume of Reagent 1 at 37°C for more than 10 minutes before use. 3. Mix all reagents thoroughly before adding them to the cuvette. 4. Start timing immediately after mixing and measure absorbance at the specified time points. 5. The blank group only needs to be measured 1-2 times. 6. For tissue samples, complete the detection within 2 hours after extraction. 7. If the sample volume is large, extract and detect the samples in batches. Technical Notes 1. FBA catalyzes the cleavage of fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. TPI converts dihydroxyacetone phosphate to glyceraldehyde-3-phosphate, and α-GPDH catalyzes the reduction of dihydroxyacetone phosphate to α-glycerophosphate using NADH, which is oxidized to NAD^+. The decrease in NADH absorbance at 340 nm is proportional to FBA activity. 2. The assay measures the decrease in absorbance (ΔA) over a 5-minute reaction period (300 seconds) at 37°C. 3. The calculation uses the NADH molar extinction coefficient $\varepsilon = 6.22 \times 10^3$ L/mol/cm and a cuvette path length d = 1 cm. 4. Four calculation methods are provided: tissue protein concentration, tissue sample weight, bacterial or cell number, and liquid sample volume. 5. Differential centrifugation with Extraction Buffers A and B is required to separate cytoplasmic and chloroplastic FBA in plant tissues. 6. Total FBA activity is recommended to be measured using the total crude-enzyme extraction protocol. For separate measurement of cytoplasmic and chloroplastic FBA, use the differential extraction protocol. Materials Required but Not Supplied UV spectrophotometer; 1 mL quartz cuvette with a 10 mm path length; mortar or homogenizer; adjustable pipette; benchtop centrifuge; constant-temperature water bath or incubator; and distilled water. Warning For Research Use Only. Not for Use in Diagnostic Procedures.

Storage	Long-term storage: -20°C. Extraction Buffer A: Store at 4°C. Extraction Buffer B: Store at 4°C. Reagent 1: Store at 4°C. Reagent 2: Store at -20°C as a powder and reconstitute before use. Reagent 3: Store at 4°C as a powder and reconstitute before use. Reagent 4: Store at 4°C as a liquid and reconstitute before use. Reagent 5: Store at -20°C as a liquid and reconstitute before use. Reconstituted Reagents 2, 3, 4, and 5 may be aliquoted and stored at -20°C for up to 1 month. Avoid repeated freeze-thaw cycles. Reagents are stable for the indicated storage period when stored under the recommended conditions.
Reconstitution	Reagent 2: Add 8.33 mL distilled water before use and mix thoroughly to dissolve. Reagent 3: Add 6 mL distilled water before use and mix thoroughly to dissolve. Reagent 4: Add 6 mL distilled water before use and mix thoroughly. Reagent 5: Add 6 mL distilled water before use and mix thoroughly. After reconstitution, aliquot and store at -20°C for up to 1 month. Avoid repeated freeze-thaw cycles.
Reaction conditions	Sample Preparation 1. Total FBA Enzyme Extraction a) Tissue: Homogenize tissue in Extraction Buffer A at a ratio of 1 g tissue to 5-10 mL buffer. The recommended ratio is 0.1 g tissue in 1 mL Extraction Buffer A. Homogenize on ice, centrifuge at 8000 × g for 10 minutes at 4°C, collect the supernatant, and keep it on ice for the assay. b) Bacteria or cells: Collect bacteria or cells by centrifugation. Resuspend 5×10^6 to 1×10^7 cells in 1 mL Extraction Buffer A; the recommended amount is 5×10^6 cells per 1 mL buffer. Sonicate on ice at 300 W using 3 seconds on and 7 seconds off for a total of 3 minutes. Centrifuge at 8000 × g for 10 minutes at 4°C, collect the supernatant, and keep it on ice for the assay. c) Liquid samples: Measure directly or dilute appropriately before the assay. 2. Cytoplasmic and Chloroplastic FBA Extraction for Plant Tissues a) Homogenize plant tissue in Extraction Buffer A at a ratio of 1 g tissue to 5-10 mL buffer. The recommended ratio is 0.1 g tissue in 1 mL Extraction Buffer A. Rapidly grind or homogenize the sample, centrifuge at 200 × g for 5 minutes at 4°C, and collect the supernatant. b) Transfer the

	supernatant from step a) to another centrifuge tube and centrifuge at 8000 × g for 10 minutes at 4°C. c) Use the supernatant from step b) for the cytoplasmic FBA activity assay. d) Resuspend the pellet from step b) in 1 mL Extraction Buffer B, vortex until dispersed, and sonicate on ice at 200 W using 3 seconds on and 7 seconds off for a total of 1 minute. Centrifuge at 8000 × g for 10 minutes at 4°C and collect the supernatant for the chloroplastic FBA activity assay. Note: Measuring total FBA activity using the total extraction protocol is recommended. Use the differential extraction protocol for separate measurement of cytoplasmic and chloroplastic FBA. Assay Protocol 1. Pre-warm the spectrophotometer for more than 30 minutes, set the wavelength to 340 nm, and zero with distilled water. 2. Pre-warm the required volume of Reagent 1 at 37°C for more than 10 minutes. 3. Add the following reagents to a 1 mL quartz cuvette in the indicated order. Blank group: 500 µL Reagent 1, 100 µL Reagent 2, 100 µL Reagent 3, 100 µL Reagent 4, 100 µL Reagent 5, and 100 µL distilled water. Do not add crude enzyme. Assay group: 500 µL Reagent 1, 100 µL Reagent 2, 100 µL Reagent 3, 100 µL Reagent 4, 100 µL Reagent 5, and 100 µL crude enzyme. Do not add distilled water. 4. Mix thoroughly and start timing immediately. Measure absorbance at 340 nm at 10 seconds total time and record the values as A_1 (Assay) and A_1 (Blank). 5. Incubate at 37°C for exactly 300 seconds (5 minutes). Measure absorbance at 340 nm at 310 seconds total time and record the values as A_2 (Assay) and A_2 (Blank). 6. Calculate ΔA (Assay) = A_1 (Assay) - A_2 (Assay); ΔA (Blank) = A_1 (Blank) - A_2 (Blank); and ΔA = ΔA (Assay) - ΔA (Blank). Note: The blank group only needs to be measured 1-2 times. Assay time: Approximately 35-40 minutes, including 30 minutes for spectrophotometer pre-warming, 10 minutes for Reagent 1 pre-warming, and 5 minutes for the reaction.
Unit definition	General Unit Definition One unit of FBA activity is defined as the amount of enzyme that catalyzes the consumption of 1 nmol NADH per minute under the assay conditions. 1. Calculation Based on Tissue Protein Concentration Unit definition: One

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	unit of FBA activity is the amount of enzyme that consumes 1 nmol NADH per minute per mg tissue protein. FBA (U/mg prot) = (321.54 × ΔA) ÷ Cpr. 2. Calculation Based on Tissue Sample Weight Unit definition: One unit of FBA activity is the amount of enzyme that consumes 1 nmol NADH per minute per g tissue. FBA (U/g) = (321.54 × ΔA) ÷ W. 3. Calculation Based on Bacterial or Cell Number Unit definition: One unit of FBA activity is the amount of enzyme that consumes 1 nmol NADH per minute per 10^4 cells. FBA (U/10^4 cells) = (321.54 × ΔA) ÷ N, where N is the bacterial or cell number expressed in units of 10^4. 4. Calculation Based on Liquid Sample Volume Unit definition: One unit of FBA activity is the amount of enzyme that consumes 1 nmol NADH per minute per mL liquid sample. FBA (U/mL) = 321.54 × ΔA. Where: V_sample is the volume of crude enzyme added to the reaction system, 0.1 mL; V_total is the total volume of crude enzyme, 1 mL; V_reaction is the total volume of the reaction system, 1 × 10^{-3} L; ε is the molar extinction coefficient of NADH, 6.22 × 10^3 L/mol/cm; d is the path length of the 1 mL quartz cuvette, 1 cm; T is the reaction time, 300 seconds = 5 minutes; Cpr is the sample protein concentration, mg/mL; W is the sample weight, g; and 10^9 is the unit-conversion factor, 1 mol = 10^9 nmol.
Usage	For Research Use Only. Not for Use in Diagnostic Procedures.

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