Sugar Beet Beta v 2 ELISA Kit

Cat. No. KITG-002 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	The kit employs a double-antibody sandwich method to measure the level of sugar beet allergen protein (Beta v 2). Microtiter plates are coated with purified antibodies to create a solid-phase antibody. Sugar beet allergen protein (Beta v 2) is added to the wells coated with monoclonal antibodies, followed by an HRP-labeled antibody to form an antibody-antigen-enzyme-labeled antibody complex. After thorough washing, the substrate TMB is added for color development. TMB is converted to blue under the catalysis of HRP enzyme and further to yellow under the action of acid. The color intensity is positively correlated with the concentration of sugar beet allergen protein (Beta v 2) in the sample. The absorbance (OD value) is measured at 450 nm using a microplate reader, and the concentration of sugar beet allergen protein (Beta v 2) in the sample is calculated from a standard curve.
Species	Beta vulgaris
Description	The kit employs a double-antibody sandwich method to measure the level of sugar be et allergen protein (Beta v 2). Microtiter plates are coated with purified antibodies to cr eate a solid-phase antibody. Sugar beet allergen protein (Beta v 2) is added to the we lls coated with monoclonal antibodies, followed by an HRP-labeled antibody to form a n antibody-antigen-enzyme-labeled antibody complex. After thorough washing, the su bstrate TMB is added for color development. TMB is converted to blue under the catal ysis of HRP enzyme and further to yellow under the action of acid. The color intensity is positively correlated with the concentration of sugar beet allergen protein (Beta v 2) in the sample. The absorbance (OD value) is measured at 450 nm using a microplate reader, and the concentration of sugar beet allergen protein (Beta v 2) in the sample i

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	s calculated from a standard curve.
Form	ELISA Kit
Applications	Measurement of sugar beet allergen protein (Beta v 2) level in specimens
Notes	Sample Handling and Requirements: 1. Serum: Collect whole blood using serum collection tubes, allow to clot at room temperature, and centrifuge at 1000×g for 15 minutes to obtain serum. Handle gently to avoid hemolysis. Test promptly or store in aliquots at -20°C or below for no more than 6 months. Thaw frozen serum samples no more than twice. 2. Plasma: Collect using EDTA or heparin tubes, centrifuge at 1000×g for 15 minutes to obtain plasma. Handle gently to avoid hemolysis. Test promptly or store as described for serum. Urine: Collect in sterile tubes, centrifuge at 2000-3000 rpm for 20 minutes, and collect the supernatant. Centrifuge again if precipitates form during storage. 3. Cell Culture Supernatant: Collect using sterile tubes for secreted components, centrifuge as above, and collect the supernatant. For intracellular components, dilute cell suspension with PBS (pH 7.2-7.4) to ~1 million cells/ml, lyse cells by freeze-thaw cycles, and centrifuge to collect the supernatant. 4. Tissue Specimens: Rinse with pre-cooled PBS (0.01M, pH=7.4), weigh, mince, and homogenize in PBS (1:9 weight/volume ratio). Further lyse cells by sonication or freeze-thaw cycles, and centrifuge at 5000×g for 5-10 minutes to collect the supernatant. Note: Extract samples as soon as possible after collection and test promptly. Store at -20°C if not testing immediately, but avoid repeated freeze-thaw cycles. Do not test samples containing NaN3 due to its inhibitory effect on HRP activity. Avoid hemolyzed or hyperlipidemic serum/plasma samples. Dilute samples with concentrations above the standard curve's upper limit before testing. Kit Components: 48-well Configuration: Instruction Manual: 1 Plate Sealer: 2 Pre-coated Assay Plate: 1×48, Storage 2-8°C Standard (16 ng/mL): 0.5 ml×1 tube, Storage 2-8°C HRP-Antibody: 3 ml×1 bottle, Storage 2-8°C Standard Diluent: 1.5 ml×1 bottle, Storage 2-8°C Sample Diluent: 3 ml×1 bottle, Storage 2-8°C Wash Buffer (20x): 20 ml×1 bottle, Storage 2-8°C TMB Substrate A: 3 ml×1 bottle, Storage 2-8°C TM

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B Substrate B: 3 ml×1 bottle, Storage 2-8°C Stop Solution: 3 ml×1 bottle, Storage 2-8°C 96-well Configuration: Instruction Manual: 1 Plate Sealer: 2 Pre-coated Assay Plate: 1×96, Storage 2-8°C Standard (16 ng/mL): 0.5 ml×1 tube, Storage 2-8°C HRP-Antibody: 6 ml×1 bottle, Storage 2-8°C Standard Diluent: 1.5 ml×1 bottle, Storage 2-8°C Sample Diluent: 6 ml×1 bottle, Storage 2-8°C Wash Buffer (20x or 30x depending on kit version): 30 ml×1 bottle (for 30x) or adjusted for 20x if applicable, Storage 2-8°C TMB Substrate A: 6 ml×1 bottle, Storage 2-8°C TMB Substrate B: 6 ml×1 bottle, Storage 2-8°C Stop Solution: 6 ml×1 bottle, Storage 2-8°C Materials Required but Not Supplied: Microplate reader (primary wavelength 450 nm, reference wavelength 630 nm) High-precision pipettes and disposable tips (0.5-10, 2-20, 20-200, 200-1000 µl) Plate washer or wash bottle 37°C incubator Dilution tubes (polypropylene) Double-distilled water, deionized water, measuring cylinders, etc. Reagent Preparation and Storage: 1. Store all kit components at 2-8°C. 2. Ensure the kit is stored under specified conditions before use. Prepare only the required amount of reagents for each experiment. 3. Centrifuge the standard vial for 30 seconds before opening to ensure all standard is concentrated at the bottom. 4. Preparation of washing solution: If crystals are present in the concentrated washing solution, heat at 37°C until all crystals dissolve. Dilute 1:19 or 1:29 with distilled water (e.g., add 190 mL of distilled water to 10 mL of concentrated washing solution and mix well). The prepared washing solution can be stored at 2-8°C for 1 week. 5. Preparation of 5 Standard Concentrations: Prepare 5 tubes. First, dilute the standard 1:1 with "standard diluent" to prepare the upper limit calibration standard S1 (e.g., mix 150 µl of standard with 150 µl of standard diluent to obtain 300 µl of S1 calibration standard). Then, add 150 µl of standard diluent to each of 4 centrifuge tubes and sequentially dilute S1 calibration standard 4 times in a 2-fold dilution series to prepare 5 calibration standards: S1, S2, S3, S4, S5. Operational Steps: 1. Sample Addition: Set up blank wells (no sample or enzyme-labeled reagent added; all other steps are the same), standard wells, and sample wells. Accurately add 50 µl of standard to the standard wells. Add 40 µl of sample diluent to the sample wells, followed by 10 µl of the sample (final dilution: 5 times). Add the sample to the bottom of the micropl

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ate well without touching the well walls and gently mix. 2. Incubation: Seal the plate wi th a plate sealer and incubate at 37°C for 30 minutes. 3. Washing: Carefully remove t he plate sealer, discard the liquid, and dry by flicking. Fill each well with washing solut ion, let stand for 30 seconds, then discard. Repeat 5 times and pat dry. 4. Enzyme Ad dition: Add 50 µl of enzyme-labeled reagent to each well except the blank well. 5. Inc ubation: Seal the plate and incubate at 37°C for 30 minutes. 6. Washing: Repeat the washing step as described in step 3. 7. Color Development: Add 50 µl of substrate A t o each well, followed by 50 µl of substrate B. Gently mix and incubate at 37°C protect ed from light for 10 minutes. 8. Termination: Add 50 µl of stop solution to each well to terminate the reaction (the color changes from blue to yellow immediately). 9. Measur ement: Set the blank well to zero and measure the absorbance (OD value) of each w ell sequentially at 450 nm. Measurement should be completed within 15 minutes after adding the stop solution. Note: Incubation time can be adjusted based on color develo pment intensity. It is recommended to observe color development intensity midway thr ough incubation. Precautions: 1. All kit components are for research purposes only! 2. The stop solution contains dilute sulfuric acid and is corrosive; handle with care. 3. Kit components contain preservatives, protein components, etc., which may cause skin a llergic reactions; wear a mask to avoid inhaling mist. 4. The color developer and conc entrated washing solution may cause skin, eye, and respiratory tract irritation; wear a mask to avoid inhaling mist. 5. Wear protective gloves, clothing, and eye and face pro tection. Wash hands thoroughly after the experiment. 6. Plate sealers are for one-time use only to avoid cross-contamination. 7. Store the substrate protected from light. 8. Do not mix kits from different batches. It is not recommended to assemble microplate strips from different plates on one plate for detection, even if they are from the same b atch, as there may be plate-to-plate variations. 9. Always prepare a standard curve in parallel for each experiment. Do not use the standard curve from a previous experime nt for calculating results of the next experiment. 10. Do not use expired kits for detecti on. 11. The substrate color developer should be colorless; if it turns blue, it has deteri orated and should not be used in the experiment. 12. Seal microplate strips with an a

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	ppropriate plate sealer for more reliable results. 13. Avoid generating bubbles during operation. 14. Do not cross-use lids from different reagent bottles. 15. Follow the instr uctions in the manual for the experiment. Failure to do so may result in changes in ex perimental results. Any experimental operations not following the manual should be c onsulted with after-sales technical support in advance. Otherwise, we do not guarante e the reliability of the experimental results. 16. All samples, washing solutions, and va rious wastes should be treated as infectious materials. Data Calculation: Dual-wavele ngth detection results do not require adjustment; proceed directly with standard curve fitting and result calculation. For single-wavelength detection results, it is recommend ed to adjust to zero using the OD value of the 0-concentration calibration standard bef ore standard curve fitting and result calculation. It is recommended to use the followin g methods for standard curve fitting: Four-parameter logistic (4-P) curve fitting: Reco mmend using concentration as the x-axis (discard the 0-concentration point) and abs orbance as the y-axis. Point-to-point fitting: Perform linear fitting separately between e ach pair of adjacent points and calculate segmentally. Other standard curve fitting me thods: Polynomial and double-logarithmic methods can also be used. Professional sof tware such as ELISACALC, SPSS, or Graphpad Prism can also be used for result fitti ng and calculation.
Storage	Store all kit components at 2-8°C. Ensure the kit is stored under specified conditions before use. Prepare only the required amount of reagents for each experiment. The prepared washing solution can be stored at 2-8°C for 1 week.
Usage	For research purposes only!

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