β-Xylosidase Activity Assay Kit

Cat. No. KITG-147 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	β-Xylosidase is widely found in plants, bacteria, and fungi and is a key enzyme that catalyzes the degradation of xylan-type hemicellulose. It plays an irreplaceable central role in the complete hemicellulose metabolic pathway. β-Xylosidase can sequentially degrade xylooligosaccharides, such as xylobiose, from the non-reducing end into xylose.
Description	β-Xylosidase catalyzes the conversion of 4-nitrophenyl-β-D-xylopyranoside to p-nitrop henol. The product has a characteristic absorption peak at 405 nm, and β-xylosidase activity can be characterized from the change in absorbance.
Form	Size 70T/25S Kit Components Extraction Solution: 30 mL × 1 bottle; store at 4°C. Reagent I: powder × 2 vials; store at -20°C protected from light. Before use, add 1.5 mL distilled water to each vial and dissolve completely. After aliquoting, Reagent I can be stored at -20°C for 2 weeks. Avoid repeated freeze-thaw cycles. Reagent II: 50 mL × 1 bottle; store at 4°C. Reagent III: 30 mL × 1 bottle; store at 4°C. Standard Solution: 1 mL × 1 vial; 10 μmol/mL p-nitrophenol standard solution; store at 4°C protected from light.
Bio-activity	Function Catalyzes the degradation of xylan-type hemicellulose and sequentially hydr olyzes xylooligosaccharides, such as xylobiose, from the non-reducing end into xylos e. In this assay, β-xylosidase catalyzes the conversion of 4-nitrophenyl-β-D-xylopyran oside to p-nitrophenol.
Applications	Applications Treatment of industrial and agricultural residues; medical supplies;

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	fibrous feed; food and paper industries. Detection Method Visible spectrophotometry; measurement wavelength: 405 nm. Compatible Sample Types Tissue; bacteria; cells; liquid samples.
Notes	Notes 1. Reagent I has a short validity period after preparation. To facilitate assay scheduling, one additional vial of Reagent I is provided as a backup. Each vial is sufficient for the determination of at least 25 samples. 2. If A(test) or ΔA(test) exceeds the linear absorbance range of the standards: if the value is higher than the highest value, appropriately dilute the crude enzyme solution with distilled water before repeat determination; if the value is lower than the lowest value, appropriately extend the enzymatic reaction time (react accurately at 45°C for 20 min; the reaction may be extended to more than 60 min) or prepare a higher-concentration sample, and modify the calculation accordingly. 3. To ensure accurate results and avoid reagent loss, read the instructions carefully before determination. The instructions actually received shall prevail. Confirm that reagent storage and preparation are adequate and that the operating procedure is clear. Be sure to select 2–3 samples with expected large differences for preliminary determination. If any problem occurs during the process, contact staff promptly. Scientific Background β-Xylosidase is widely found in plants, bacteria, and fungi and is a key enzyme in the degradation of xylan-type hemicellulose. It plays an irreplaceable central role in the complete hemicellulose metabolic pathway and can sequentially degrade xylooligosaccharides, such as xylobiose, from the non-reducing end into xylose. Features & Benefits The assay uses 4-nitrophenyl-β-D-xylopyranoside as the substrate. The reaction product, p-nitrophenol, has a characteristic absorption peak at 405 nm, allowing β-xylosidase activity to be determined from the change in absorbance. Handling Store at -20°C. Protect Reagent I and the Standard Solution from light. After reconstitution, aliquot Reagent I and avoid repeated freeze-thaw cycles. Warning For Research Use Only. Not for Use in Diagnostic Procedures.
Storage	Storage Overall storage temperature: -20°C. Extraction Solution: 4°C. Reagent I: -20°C, protected from light. After reconstitution, aliquot and store at -20°C. Reagent II:

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	4°C. Reagent III: 4°C. Standard Solution: 4°C, protected from light. Stability After reconstitution, aliquoted Reagent I can be stored at -20°C for 2 weeks. Avoid repeated freeze-thaw cycles.
Reaction conditions	Preparation Reagent I reconstitution: Before use, add 1.5 mL distilled water to each vial of Reagent I and dissolve completely. After aliquoting, the reconstituted reagent can be stored at -20°C for 2 weeks. Avoid repeated freeze-thaw cycles. Preparation of standard dilutions (prepare immediately before use): Dilute the 10 μmol/mL p-nitrophenol standard solution with Reagent II. First prepare a 1.0 μmol/mL standard solution by mixing 100 μL standard solution with 900 μL Reagent II. Prepare 0.3, 0.2, and 0.1 μmol/mL standard dilutions by mixing 300, 200, and 100 μL of the 1.0 μmol/mL standard solution with 700, 800, and 900 μL Reagent II, respectively. Prepare 0.05, 0.025, and 0.0125 μmol/mL standard dilutions by 1:1 serial dilution of the 0.1, 0.05, and 0.025 μmol/mL solutions with Reagent II; mix 500 μL of each preceding solution with 500 μL Reagent II. Assay Protocol 1. Preparation of crude enzyme solution: The sample amount and ratio may be adjusted appropriately according to the results of a preliminary experiment. Tissue: Process the sample at a tissue mass (g) to Extraction Solution volume (mL) ratio of 1:(5–10). It is recommended to weigh 0.1 g tissue and add 1 mL Extraction Solution. Homogenize on ice and centrifuge at 12,000 g for 10 min at 4°C. Transfer the supernatant to ice for determination. Bacteria or cells: Collect bacteria or cells by centrifugation in a centrifuge tube. Process the sample at a bacteria or cell count (10^4 cells) to Extraction Solution volume (mL) ratio of (500–1000):1. It is recommended to add 1 mL Extraction Solution to 5 × 10^6 bacteria or cells. Perform ultrasonic disruption on ice at 200 W for 3 s with a 10 s interval, repeated 30 times. Centrifuge at 12,000 g for 10 min at 4°C and transfer the supernatant to ice for determination. Liquid samples: Determine directly or after appropriate dilution. 2. Measurement procedure: Preheat the spectrophotometer for more than 30 min, set the wavelength to 405 nm, and zero with distilled water. Add the

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	following reagents sequentially to centrifuge tubes: Test tube: 200 μL crude enzyme solution, 50 μL Reagent I, and 350 μL Reagent II. Control tube: 200 μL crude enzyme solution and 400 μL Reagent II. Standard tube: 200 μL standard dilution and 400 μL Reagent II. Blank tube: 600 μL Reagent II. Mix thoroughly and react accurately at 45°C for 20 min. Seal the tubes to prevent water loss and cool to room temperature. Add 400 μL Reagent III to each tube, mix thoroughly, and react at room temperature for 5 min. Transfer the reaction solution to a 1 mL glass cuvette and measure the absorbance at 405 nm. Record the values as A(test), A(control), A(standard), and A(blank). Calculate ΔA(test) = A(test) − A(control), and ΔA(standard) = A(standard) − A(blank). Set up one control tube for each sample. Determine each concentration of the standard tubes and the blank tube only 1–2 times. Assay time Accurate reaction at 45°C for 20 min, followed by reaction at room temperature for 5 min. Analysis Standard curve: Use 0.3, 0.2, 0.1, 0.05, 0.025, and 0.0125 μmol/mL as the x-axis coordinates and the corresponding ΔA(standard) values as the y-axis coordinates to prepare a standard curve. Obtain the standard equation y = kx + b. Substitute ΔA(test) into the equation to calculate x (μmol/mL). Calculation by tissue protein concentration: β-Xylosidase (U/mg prot) = (x × D)/(Cpr × T) = (0.05 × x × D)/Cpr Calculation by tissue sample mass: β-Xylosidase (U/g) = (x × V_{total} × D)/(W × T) = (0.05 × x × D)/W Calculation by bacteria or cell count: β-Xylosidase (U/10^4 cells) = (x × V_{total} × D)/(bacteria or cell count × T) = (0.05 × x × D)/(bacteria or cell count) Calculation by liquid sample volume: β-Xylosidase (U/mL) = (x × D)/T = 0.05 × x × D Definitions: V_{total}: total crude enzyme solution volume, 1 mL; Cpr: sample protein concentration, mg/mL; W: sample mass, g; bacteria or cell count: expressed in units of 10^4, so if there are 5 × 10^6 bacteria or cells, enter 500; T: enzymatic reaction time, 20 min; D: dilution factor of the crude enzyme solution, or 1 if not diluted.
Unit definition	By tissue protein concentration: one unit is defined as the amount of enzyme that generates 1 μmol p-nitrophenol per minute per mg of tissue protein. By tissue sample

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	mass: one unit is defined as the amount of enzyme that generates 1 μmol p-nitrophenol per minute per g of tissue sample. By bacteria or cell count: one unit is defined as the amount of enzyme that generates 1 μmol p-nitrophenol per minute per 10^4 bacteria or cells. By liquid sample volume: one unit is defined as the amount of enzyme that generates 1 μmol p-nitrophenol per minute per mL of liquid sample.
Usage	Materials Required but Not Supplied Visible spectrophotometer 1 mL glass cuvette (p ath length 10 mm, slit 3 mm, volume 1.05 mL) Mortar or homogenizer Adjustable pipe tte Benchtop centrifuge Constant-temperature water bath or incubator Distilled water

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