β-Glucosidase (β-GC) Activity Assay Kit

Cat. No. KITG-026 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	β-Glucosidase (β-GC) is widely present in animals, plants, microorganisms, and cultured cells, catalyzing the hydrolysis of β-glycosidic bonds, and has multiple physiological functions: in the saccharification of cellulose, β-GC is responsible for further hydrolysis of cellobiose and cello-oligosaccharides to generate glucose; β-GC hydrolyzes terpenoid aroma precursors, converting glycosidic bound states into free states, thereby producing aroma substances.
Species	Animals, Plants, Microorganisms, Cultured cells
Description	β-Glucosidase (β-GC) can decompose p-nitrophenyl-β-D-glucopyranoside to generat e p-nitrophenol. The product has a characteristic absorption peak at 400 nm, and the activity of β-glucosidase can be characterized by the change in absorbance at 400 n m.
Form	Colorimetric Activity Assay Kit (Visible Spectrophotometry)
Applications	Detection of β-Glucosidase (β-GC) activity in animal tissue, plant tissue, microorganisms, cultured cells, serum, plasma, and culture medium. For research use only. Not for use in diagnostic procedures.
Notes	Kit Components: Extraction Buffer: Liquid 120 mL × 1 bottle, store at 4°C. Reagent I: Powder × 2 bottles, store at -20°C. Add 10 mL distilled water to each bottle and dissolve completely before use. Reagent II: Liquid 25 mL × 1 bottle, store at 4°C. Reagent III: Liquid 15 mL × 1 bottle, store at 4°C. Standard Solution: Liquid 1 mL × 1

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vial, store at 4°C. 10 μmol/mL p-nitrophenol standard solution. Standard Preparation: Preparation of Standard Dilution Solution: Dilute the 10 μmol/mL p-nitrophenol standard solution with distilled water to prepare concentrations of 300, 200, 100, 50, 25, and 12.5 nmol/mL. Standard Dilution Table: No. 1: 100 μL Standard solution + 900 μL Distilled water = 1000 nmol/mL No. 2: 100 μL Standard solution + 700 μL Distilled water = 300 nmol/mL No. 3: 100 μL Standard solution + 800 μL Distilled water = 200 nmol/mL No. 4: 100 μL Standard solution + 900 μL Distilled water = 100 nmol/mL No. 5: 50 μL Standard solution + 500 μL Distilled water = 50 nmol/mL No. 6: 50 μL Standard solution + 500 μL Distilled water = 25 nmol/mL No. 7: 50 μL Standard solution + 500 μL Distilled water = 12.5 nmol/mL Sample Preparation: Instruments and reagents required for the assay: Visible spectrophotometer/microplate reader, micro glass cuvette/96-well plate, mortar/homogenizer, adjustable pipette, benchtop centrifuge, constant temperature water bath/incubator, and distilled water. Preparation of Crude Enzyme Solution (The sample volume and ratio can be appropriately adjusted according to the pre-experimental results): 1. Bacteria or cells: Centrifuge to collect bacteria or cells into a centrifuge tube. Process the sample at a ratio of bacterial or cell number (10^4 cells): extraction buffer volume (mL) = (500–1000):1 (It is recommended to add 1 mL extraction buffer per 10 million bacteria or cells). Sonicate on ice (power 20% or 200 W, sonication 3 s, interval 10 s, repeat 30 times), centrifuge at 4°C 15000 g for 20 min, and place the supernatant on ice for assay. 2. Tissue: Process the sample at a ratio of tissue mass (g): extraction buffer volume (mL) = 1:(5–10) (It is recommended to weigh 0.2 g tissue and add 1 mL extraction buffer). Homogenize on ice, centrifuge at 4°C 15000 g for 20 min, and place the supernatant on ice for assay. 3. Serum (plasma), culture medium and other liquid samples: Detect directly or dilute appropriately before detection. Assay Procedure: 1. Preheat the spectrophotometer/microplate reader for more than 30 min, adjust the wavelength to 400 nm, and zero with distilled water. 2. Add the following

reagents sequentially into the centrifuge tube: Test Group (μL): Reagent I 160, Reagent II 200, Crude enzyme solution 40 Control Group (μL): Reagent I 160, Reagent II 200, Crude enzyme solution 40 Standard Group (μL): — Blank Group (μL): — Mix thoroughly, react accurately at 37°C for 30 min, immediately place in boiling water bath for 5 min (seal to prevent water loss), cool to room temperature with running water. Add Reagent I: 160 μL to each tube. Mix thoroughly, centrifuge at 4°C 8000 g for 5 min, take the supernatant. Add the following reagents sequentially into a 96-well plate or micro glass cuvette: - Supernatant: 60 μL (Test and Control) - Standard dilution: 60 μL (Standard) - Distilled water: 60 μL (Blank) - Reagent III: 120 μL (all groups) Mix thoroughly, stand at room temperature for 2 min. Absorbance measurement: Measure the absorbance at 400 nm, recorded as A_Test, A_Control, A_Standard and A_Blank; Calculate ΔA_Test = A_Test – A_Control, ΔA_Standard = A_Standard – A_Blank. Note: Each test group needs to set up a control group, and the blank group only needs to be measured 1–2 times. Establishment of standard curve: Use 300, 200, 100, 50, 25, and 12.5 nmol/mL as the abscissa (x), and the corresponding ΔA_Standard as the ordinate (y) to plot the standard curve, obtain the standard equation y = kx + b, substitute ΔA_Test into the formula to obtain x (nmol/mL). Calculations: 1. Calculated by tissue sample protein concentration: Unit definition: One unit of enzyme activity is defined as the amount of enzyme that generates 1 nmol of p-nitrophenol per mg of tissue protein per hour. β-GC (U/mg prot) = (x × V_RT) / (V_S × Cpr × T) = (20 × x) / Cpr 2. Calculated by tissue sample mass: Unit definition: One unit of enzyme activity is defined as the amount of enzyme that generates 1 nmol of p-nitrophenol per g of tissue per hour. β-GC (U/g) = (x × V_RT × V_ST) / (V_S × W × T) = (20 × x) / W 3. Calculated by bacteria or cell number: Unit definition: One unit of enzyme activity is defined as the amount of enzyme that generates 1 nmol of p-nitrophenol per 10,000 bacteria or cells per hour. β-GC (U/10^4 cell) = (x × V_RT × V_ST) / (V_S × Number of bacteria or cells × T) = (20 × x) / Number of bacteria or cells 4. Calculated by liquid sample volume: Unit definition: One unit of enzyme activity is defined as the amount of enzyme that

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	generates 1 nmol of p-nitrophenol per mL of liquid sample per hour. β-GC (U/L) = (x × V_RT) / (V_S × T) = 20 × x Notes: V_S (Sample): Volume of crude enzyme solution added to the reaction system, 0.04 mL; V_ST (Total sample): Total volume of crude enzyme solution, 1 mL; V_RT (Total reaction): Total volume of reaction system, 0.4 mL; Cpr: Protein concentration of sample, mg/mL; W: Sample mass, g; Number of cells or bacteria: in units of 10,000; T: Reaction time, 0.5 h. Precautions: 1. If the measured absorbance exceeds the linear range of the standard absorbance, it is recommended to appropriately increase the sample volume or dilute the crude enzyme solution before measurement, and modify accordingly in the calculation. 2. To ensure accurate results and avoid reagent loss, please read the instructions carefully before measurement, confirm whether reagent storage and preparation are adequate, whether the operation steps are clear, and be sure to take 2–3 samples with expected large differences for pre-assay.
Storage	Store at -20°C. Size: 120T/50S.
Shipping	Shipped on dry ice or blue ice.
Reaction conditions	One unit of enzyme activity is defined as the amount of enzyme that generates 1 nmol of p-nitrophenol per mg of tissue protein per hour (or per g tissue per hour, or per 10,000 bacteria or cells per hour, or per mL of liquid sample per hour, depending on sample type)
EC	Extraction Buffer (4°C)
CAS	Boiling water bath 5 min (to stop reaction)
Unit definition	One unit of enzyme activity is defined as the amount of enzyme that generates 1 nmol of p-nitrophenol per mg of tissue protein per hour (or per g tissue per hour, or per 10,000 bacteria or cells per hour, or per mL of liquid sample per hour, depending

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	on sample type)
Storage Buffer	Extraction Buffer (4°C)
Heat inactivation	Boiling water bath 5 min (to stop reaction)
Labeling Efficiency	For research use only. Not for use in diagnostic procedures.
Usage	For research use only. Not for use in diagnostic procedures.

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