β-Glucosidase (β-GC) Activity Assay Kit, Micro Method

Product Information

Product code: 111990

β-Glucosidase (β-GC, EC 3.2.1.21) is widely found in animals, plants, microorganisms, and cultured cells. It catalyzes the hydrolysis of β-glycosidic bonds and has multiple physiological functions.

In cellulose saccharification, β-GC further hydrolyzes cellobiose and cellooligosaccharides to produce glucose. It can also hydrolyze terpene aroma precursors from the glycosidically bound state to the free state, producing aroma. In plants, β-GC can hydrolyze prunasin and release HCN, helping prevent insect feeding.

This assay is based on the decomposition of p-nitrophenyl-β-D-glucopyranoside by β-GC to generate p-nitrophenol, which has a maximum absorption peak at 400 nm. β-GC activity is calculated by measuring the rate of increase in absorbance.

Package Contents

Code	Component	Amount	Storage
111990.1	Reagent I	18 mg	-20°C, protected from light
111990.2	Reagent II	15 mL	2-8°C
111990.3	Reagent III	15 mL	2-8°C
111990.4	Extraction Solution	100 mL	2-8°C
111990.m	Instruction Manual	1 copy	-

Quality and Safety Information

Component	Quality Standard	Main Toxicity
Reagent I	--	--
Reagent II	--	--
Reagent III	--	--
Extraction Solution	--	--

Transportation and Storage

Transportation	Transport with ice packs.
Storage	Store each component according to the instructions above.
Shelf life	180 days.

Instructions for Use

1. Preparation of Crude Enzyme Extract

1.1 Bacteria or Cultured Cells

1. Collect bacteria or cells into a centrifuge tube.
2. Centrifuge and discard the supernatant.
3. Add extraction solution according to a bacteria or cell number of 500-1000 × 10^4cells per 1 mL extraction solution. It is

recommended to add 500×10^4 bacteria or cells to 1 mL extraction solution.
4. Disrupt the bacteria or cells by ultrasonication in an ice bath at 20% power or 200 W: ultrasound for 3 s, interval for 10 s, repeat 30 times.
5. Centrifuge at 15000 g and 4°C for 10 min.
6. Collect the supernatant and keep it on ice until testing.

1.2 Tissue

1. Add extraction solution according to a tissue mass (g): extraction solution volume (mL) ratio of 1:5-10. It is recommended to weigh about 0.1 g tissue and add 1 mL extraction solution.
2. Homogenize in an ice bath.
3. Centrifuge at 15000 g and 4°C for 10 min.
4. Collect the supernatant and keep it on ice until testing.

1.3 Liquid Samples

Liquid samples such as culture medium and serum or plasma can be tested directly.

2. Reagent Preparation

1. Before use, add 12 mL distilled water to each bottle of Reagent I.
2. Dissolve thoroughly before use.
3. Store unused Reagent I at -20°C.

3. Assay Procedure

1. Preheat the spectrophotometer or microplate reader for at least 30 min.
2. Set the wavelength to 400 nm.
3. Zero the instrument with distilled water.

3.1 Sample Loading

Component	Assay Tube	Control Tube
Reagent I	120 µL	-
Distilled water	-	120 µL
Reagent II	150 µL	150 µL
Sample	30 µL	30 µL

1. Mix thoroughly.
2. Incubate in a 37°C constant-temperature water bath for 30 min.
3. Immediately place in a 95°C water bath for 5 min. Cap tightly to prevent water loss.
4. Cool under running water and mix thoroughly to ensure the concentration remains unchanged.
5. Centrifuge at 8000 g and 4°C for 5 min.
6. Collect the supernatant and add the following reagents to an EP tube or 96-well plate.

Component	Assay Tube	Control Tube
Supernatant	70 µL	70 µL
Reagent III	130 µL	130 µL

1. Mix thoroughly and let stand at room temperature for 2 min.
2. Measure the absorbance at 400 nm, recorded as A.
3. Calculate $\Delta A = A_{assay} - A_{control}$.

Each assay tube requires one corresponding control tube.

β-GC Activity Calculation

4.1 Calculation Using a Micro Quartz Cuvette

The regression equation measured under standard conditions is y = 0.00585x - 0.0027, where x is the standard concentration in nmol/mL and y is the absorbance value.

4.1.1 Calculated by Liquid Volume

Unit definition: The amount of sample in mL that produces 1 nmol p-nitrophenol per minute is defined as one enzyme activity unit.

β-GC activity (nmol/min/mL) = $[(\Delta A + 0.0027) \div 0.00585 \times V_{total\ reaction}] \div V_{sample} \div T = 56.98 \times (\Delta A + 0.0027)$

4.1.2 Calculated by Sample Protein Concentration

Unit definition: The amount of tissue protein in mg that produces 1 nmol p-nitrophenol per minute is defined as one enzyme activity unit.

β-GC activity (nmol/min/mg prot) = $[(\Delta A + 0.0027) \div 0.00585 \times V_{total\ reaction}] \div (V_{sample} \times Cpr) \div T = 56.98 \times (\Delta A + 0.0027) \div Cpr$

4.1.3 Calculated by Sample Fresh Weight

Unit definition: The amount of fresh tissue in g that produces 1 nmol p-nitrophenol per minute is defined as one enzyme activity unit.

β-GC activity (nmol/min/g fresh weight) = $[(\Delta A + 0.0027) \div 0.00585 \times V_{total\ reaction}] \div (W \times V_{sample} \div V_{total\ sample}) \div T = 56.98 \times (\Delta A + 0.0027) \div W$

4.1.4 Calculated by Bacterial or Cell Density

Unit definition: The amount of 10^4 bacteria or cells that produces 1 nmol p-nitrophenol per minute is defined as one enzyme activity unit.

β-GC activity (nmol/min/10^4cells) = $[(\Delta A + 0.0027) \div 0.00585 \times V_{total\ reaction}] \div (500 \times V_{sample} \div V_{total\ sample}) \div T = 0.114 \times (\Delta A + 0.0027)$

4.2 Calculation Using a 96-Well Plate

The regression equation measured under standard conditions is y = 0.0039x - 0.0027, where x is the standard concentration in nmol/mL and y is the absorbance value.

4.2.1 Calculated by Liquid Volume

Unit definition: The amount of sample in mL that produces 1 nmol p-nitrophenol per minute is defined as one enzyme activity unit.

β-GC activity (nmol/min/mL) = $[(\Delta A + 0.0027) \div 0.0039 \times V_{total\ reaction}] \div V_{sample} \div T = 85.47 \times (\Delta A + 0.0027)$

4.2.2 Calculated by Sample Protein Concentration

Unit definition: The amount of tissue protein in mg that produces 1 nmol p-nitrophenol per minute is defined as one enzyme activity unit.

β-GC activity (nmol/min/mg prot) = $[(\Delta A + 0.0027) \div 0.0039 \times V_{total\ reaction}] \div (V_{sample} \times Cpr) \div T = 85.47 \times (\Delta A + 0.0027) \div Cpr$

4.2.3 Calculated by Sample Fresh Weight

Unit definition: The amount of fresh tissue in g that produces 1 nmol p-nitrophenol per minute is defined as one enzyme activity unit.

β-GC activity (nmol/min/g fresh weight) = $[(\Delta A + 0.0027) \div 0.0039 \times V_{total\ reaction}] \div (W \times V_{sample} \div V_{sample\ total}) \div T = 85.47 \times (\Delta A + 0.0027) \div W$

4.2.4 Calculated by Bacterial or Cell Density

Unit definition: The amount of 10,000 bacteria or cells that produces 1 nmol p-nitrophenol per minute is defined as one enzyme activity unit.

β-GC activity (nmol/min/10^4cells) = $[(\Delta A + 0.0027) \div 0.0039 \times V_{reaction\ total}] \div (500 \times V_{sample} \div V_{sample\ total}) \div T = 0.171 \times (\Delta A + 0.0027)$

Calculation Parameters

$V_{reaction\ total}$	Total volume of the reaction system, 0.3 mL
V_{sample}	Sample volume added to the reaction system, 0.03 mL
$V_{sample\ total}$	Volume of extraction solution added, 1 mL
Cpr	Sample protein concentration, mg/mL
W	Sample mass, g
500	Total number of cells or bacteria, 500×10^4
T	Reaction time, 30 min

Precautions

1. This 100T kit can test 48 samples.
2. Self-prepared supplies include a spectrophotometer or microplate reader, 1 mL cuvette or 96-well plate, water bath, adjustable pipette, mortar, ice, and distilled water.
3. Before formal measurement, select 2-3 samples with large expected differences for preliminary measurement.
4. If the measured ΔA is less than 0.01, the 37°C reaction time can be extended. If the measured ΔA is greater than 1.5, dilute the sample with extraction solution before measurement and modify the calculation formula accordingly.
5. To ensure accurate results and avoid reagent loss, read the instruction manual carefully before measurement. The contents of the manual actually received shall prevail.
6. This product is intended only for scientific research by professionals. It must not be used for clinical diagnosis or treatment, must not be used in food or medicine, and must not be stored in ordinary residences.
7. For safety and health, wear a lab coat and disposable gloves during operation.