β-Glucosidase (β-GC) Activity Assay Kit

Product Information

Product Code	111989
Method	Spectrophotometric method
Specification	50T

Product Introduction

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.

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

This assay measures β-GC activity by detecting the breakdown of p-nitrophenyl-β-D-glucopyranoside to produce p-nitrophenol, which has a maximum absorbance peak at 400 nm. β-GC activity is calculated from the rate of increase in absorbance.

Performance may vary under different assay conditions and with different detection instruments. Reference data are for guidance only.

Package Contents and Storage

Code	Component	Quantity	Storage
111989.1	Reagent 1	2 x 15 mg	-20°C, protected from light
111989.2	Reagent 2	25 mL	2-8°C
111989.3	Reagent 3	50 mL	2-8°C
111989.4	Extraction Solution	50 mL	2-8°C
111989.m	Manual	1 copy	-

Quality and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent 1	--	--
Reagent 2	--	--
Reagent III	--	--
Extraction Solution	--	--

Transportation and Storage

The product is transported with ice packs.

Store each component according to the storage conditions listed above. Shelf life is 180 days.

Instructions for Use

1. Preparation of Crude Enzyme Extract

Bacteria or Cultured Cells

1. Collect bacteria or cells into a centrifuge tube, centrifuge, and discard the supernatant.
2. Add Extraction Solution at a ratio of bacteria or cells to Extraction Solution volume of 500-1000:1, where the cell number is expressed as 10^4cells and the volume is expressed in mL. The recommended ratio is 5,000,000 bacteria or cells with 1 mL Extraction Solution.
3. Ultrasonically disrupt the bacteria or cells in an ice bath using 20% power or 200 W, ultrasound for 3 s, interval for 10 s, and repeat 30 times.
4. Centrifuge at 15000 g and 4°C for 10 min.
5. Collect the supernatant and keep it on ice until testing.

Tissue

1. Add Extraction Solution at a tissue mass to Extraction Solution volume ratio of 1:5-10, using g for tissue mass and mL for volume. The recommended preparation is approximately 0.1 g tissue with 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.

Culture Medium, Serum, Plasma, and Other Liquid Samples

Test directly.

2. Reagent Preparation

Before use, add 10 mL distilled water to each bottle of Reagent I and dissolve thoroughly. Store unused prepared reagent at -20°C.

3. Assay Procedure

1. Preheat the spectrophotometer for more than 30 min.
2. Set the wavelength to 400 nm.
3. Use distilled water to zero the instrument.

Sample Addition

Component	Assay Tube	Control Tube
Reagent I	400 μL	-
Distilled Water	-	400 μL
Reagent II	500 μL	500 μL
Sample	100 μL	100 μL

1. Mix thoroughly and place in a 37°C water bath for exactly 30 min.
2. Immediately place in a 95°C water bath for 5 min. Tighten the cap to prevent water loss.
3. Cool under running water, then mix thoroughly to ensure the concentration remains unchanged.
4. Centrifuge at 8000 g and 4°C for 5 min.
5. Collect the supernatant and add the following reagents to an EP tube.

Component	Assay Tube	Control Tube
Supernatant	500 μL	500 μL
Reagent III	1000 μL	1000 μL

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

> Each assay tube requires one control tube.

β-GC Activity Calculation

The regression equation measured under standard conditions is:

y = 0.00543x - 0.0027

In this equation, x is the standard concentration in nmol/mL, and y is the absorbance value.

Calculated by Liquid Volume

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

β-GC activity (nmol/min/mL) = [(ΔA + 0.0027) ÷ 0.00543 x $V_{total\ reaction}$] ÷ V_{sample}÷ T = 61.39 x (ΔA + 0.0027)

Calculated by Sample Protein Concentration

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

β-GC activity (nmol/min/mg prot) = [(ΔA + 0.0027) ÷ 0.00543 x $V_{total\ reaction}$] ÷ (V_{sample}x Cpr) ÷ T = 61.39 x (ΔA + 0.0027) ÷ Cpr

Calculated by Sample Fresh Weight

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

β-GC activity (nmol/min/g fresh weight) = [(ΔA + 0.0027) ÷ 0.00543 x $V_{total\ reaction}$] ÷ (W x V_{sample}÷ $V_{total\ sample}$) ÷ T = 61.39 x (ΔA + 0.0027) ÷ W

Calculated by Bacterial or Cell Density

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

β-GC activity (nmol/min/10^4cells) = [(ΔA + 0.0027) ÷ 0.00543 x $V_{total\ reaction}$] ÷ (500 x V_{sample}÷ $V_{total\ sample}$) ÷ T = 0.123 x (ΔA + 0.0027)

Formula Parameters

Parameter	Definition
$V_{total\ reaction}$	Total volume of the reaction system, 1 mL
V_{sample}	Sample volume added to the reaction system, 0.1 mL
$V_{total\ sample}$	Volume of extract added, 1 mL
Cpr	Sample protein concentration, mg/mL
W	Sample mass, g
500	Total number of cells or bacteria, 5,000,000
T	Reaction time, 30 min

Precautions

1. This 50T product can test 24 samples.
2. Materials required but not provided: spectrophotometer, 1 mL cuvette, water bath, adjustable pipette, mortar, ice, and distilled water.
3. Before formal measurement, use 2-3 samples with large expected differences for preliminary testing.
4. If the measured ΔA is less than 0.01, the reaction time at 37°C can be extended.
5. If the measured ΔA is greater than 1.5, dilute the sample with Extraction Solution before measurement. Modify the calculation formula accordingly.

6. Read the instructions carefully before measurement to ensure accurate results and avoid reagent loss.
7. This product is for scientific research by professionals only. It must not be used for clinical diagnosis or treatment, must not be used in food or drugs, and must not be stored in ordinary residences.
8. For safety and health, wear a lab coat and disposable gloves when handling.