β-Glucosidase (β-GC) Activity Assay Kit - Microplate Method

Product Information

Product Code	111992
Assay Size	100T
Detection Method	Microplate method

Product Introduction

β-Glucosidase (β-GC, EC 3.2.1.21) is widely present 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 cello-oligosaccharides to produce glucose. β-GC also hydrolyzes terpene aroma precursors, converting glycosidically bound forms into free forms and producing aroma. In plants, β-GC can hydrolyze prunasin and release HCN, helping prevent insect feeding.

β-GC decomposes p-nitrophenyl-β-D-glucopyranoside to produce p-nitrophenol, which has a maximum absorption peak at 400 nm. β-GC activity is calculated by measuring the rate of increase in absorbance.

Product Packing List

Code	Component	Quantity
111992.1	Reagent I	1 bottle
111992.2	Reagent II	1 bottle
111992.3	Reagent III	1 bottle
111992.4	Extraction Solution	1 bottle
111992.m	Instruction Manual	1 copy

Quality Standards and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent I	--	--
Reagent II	--	--
Reagent III	--	--
Extraction Solution	--	--

Transportation and Storage

Transportation	Transport with ice packs.
Storage	Store Reagent I at -20°C. Store the remaining components at 2-8°C.
Shelf Life	180 days

Instructions for Use

1. Preparation of Crude Enzyme Extract

1.1 Bacteria or Cultured Cells

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

1.2 Tissue

1. Add Extraction Solution according to the ratio of tissue mass (g) to Extraction Solution volume (mL) at 1:5-10. It is recommended to weigh approximately 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 for testing.

1.3 Liquid Samples

Culture medium, serum, plasma, and other liquid samples can be tested directly.

2. Reagent Preparation

Before use, add 12 mL distilled water to each bottle of Reagent I and dissolve thoroughly. Store unused prepared Reagent I at -20°C.

3. Assay Procedure

1. Preheat the microplate reader for at least 30 min.
2. Set the wavelength to 400 nm and zero with distilled water.
3. Add reagents according to the table below.

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

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. Cap tightly to prevent water loss.
3. Cool under running water and 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 a 96-well plate.

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

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

> Each assay tube requires one control tube.

β-GC Activity Calculation

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

4.1 Calculation by Liquid Volume

Unit definition: the production of 1 nmol p-nitrophenol per mL sample per minute is defined as one unit of enzyme activity.

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 Calculation by Sample Protein Concentration

Unit definition: the production of 1 nmol p-nitrophenol per mg tissue protein per minute is defined as one unit of enzyme activity.

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

4.3 Calculation by Sample Fresh Weight

Unit definition: the production of 1 nmol p-nitrophenol per g tissue per minute is defined as one unit of enzyme activity.

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

4.4 Calculation by Bacterial or Cell Density

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

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

Formula Parameters

Parameter	Description
$V_{reaction\ total}$	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, 5 million
T	Reaction time, 30 min

Precautions

1. This 100T kit can test 48 samples.