

**$\beta$ -Glucosidase ( $\beta$ -GC) Activity Assay Kit, Micro Method****Product Information****Product code:** 111990

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

In cellulose saccharification,  $\beta$ -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,  $\beta$ -GC can hydrolyze prunasin and release HCN, helping prevent insect feeding.

This assay is based on the decomposition of p-nitrophenyl- $\beta$ -D-glucopyranoside by  $\beta$ -GC to generate p-nitrophenol, which has a maximum absorption peak at 400 nm.  $\beta$ -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  $500-1000 \times 10^4$  cells per 1 mL extraction solution. It is

- recommended to add  $500 \times 10^4$  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  $\Delta A = A_{\text{assay}} - A_{\text{control}}$ .

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.

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

#### 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.

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

#### 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.

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

#### 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.

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

### 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.

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

#### 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.

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

#### 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.

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

#### 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.

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

### Calculation Parameters

|                             |  |
|-----------------------------|--|
| $V_{\text{reaction total}}$ | Total volume of the reaction system, 0.3 mL          |
| $V_{\text{sample}}$         | Sample volume added to the reaction system, 0.03 mL  |
| $V_{\text{sample total}}$   | Volume of extraction solution added, 1 mL            |
| Cpr                         | Sample protein concentration, mg/mL                  |
| W                           | Sample mass, g                                       |
| 500                         | Total number of cells or bacteria, $500 \times 10^4$ |
| 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  $\Delta A$  is less than 0.01, the 37°C reaction time can be extended. If the measured  $\Delta A$  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.