

## **$\beta$ -1,3-glucanase( $\beta$ -1,3-GA) Assay Kit**

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Microplate reader

**Catalog Number:** NA0596

**Size:**100T/48S

### **Components:**

Extraction Solution: Liquid 100 mL×1. Storage at 4°C.

Reagent I: Powder×1. Storage at 4°C. Dissolve with 3.5 mL of distilled water before use.

Reagent II: Liquid 30 mL×1. Storage at 4°C.

Standard: Powder×1. Storage at 4°C. Containing 10 mg of anhydrous glucose (dry weight loss < 0.2%). Dissolve the standard with 1 mL of distilled water to generate a 10 mg/mL glucose standard solution, store at 4°C and use within one week.

### **Product Description**

$\beta$ -1,3-GA (EC 3.2.1.73) mainly exists in plants and catalyzes the hydrolysis of  $\beta$ -1, 3-glucoside bond. A large number of  $\beta$ -1,3-GA can be induced by plant infection or other adverse conditions. Therefore,  $\beta$ -1,3-GA activity assay has been widely used in plant pathology and stress physiology studies.

$\beta$ -1,3-GA hydrolyzes laminarin and inner cuts  $\beta$ -1, 3-glucoside bond to produce reducing terminus. The enzyme activity is calculated by measuring the rate of reducing sugar production.

### **Reagents and Equipment Required but Not Provided.**

Spectrophotometer/microplate reader, desk centrifuge, adjustable pipette, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, lilled water.

### **Procedure:**

#### **I. Sample Extraction:**

1. Tissue sample:

According to the ratio of tissue weight(g) and extract solution volume(mL) is 1:5~10 (It is recommended to add 1 mL of Extract solution to 0.1 g of tissue) for ice bath homogenization. Centrifuge at 12000 ×g for 10 minutes at 4°C to remove insoluble materials and take the supernatant on ice before test.

2. Bacteria or cells:

Collecting bacteria or cells into the centrifuge tube, after centrifugation discard supernatant. According to the ratio of Bacteria or cell amount ( $10^4$ ) and Extract solution volume(mL) is 500~1000:1 for ice bath homogenization. It is recommended to 5 million of bacteria or cells with 1 mL of Extract Solution. Use ultrasonic to splitting bacteria and cell (placed on ice, 200W, work time 3s, interval 10s, repeat for 30 times). Centrifuge at 12000 ×g for 10 minutes at 4°C to remove insoluble materials and take the supernatant on ice before test.

## II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader 30 minutes, adjust wavelength to 540 nm, set zero with distilled water.
2. Standard preparation: Dilute the 10 mg/mL glucose standard solution 1, 0.8, 0.6, 0.4, 0.2 mg/mL with distilled water.
3. Add reagents to 1.5 mL EP tube with the following list:

Reagent ( $\mu\text{L}$ )	Test tube (T)	Control tube (C)	Standard tube (S)	Blank tube (B)
Sample	35	35		
Standard Solution			35	
Distilled water		35	35	70
Reagent I	35			
Mix thoroughly, put in 37°C water bath for 60 minutes				
Reagent II	230	230	230	230

Mix thoroughly, boiling water bath for 5 minutes (cover tightly to prevent water loss), add 200  $\mu\text{L}$  to micro glass cuvette/96 well flat-bottom plate, detect the absorbance after cooling with running water.  $\Delta A = A(T) - A(C)$ ,  $A = A(S) - A(B)$ . If the absorbance is great than 2, dilute sample with Extract solution, multiplied the dilution ratio in the calculation formula.

## III. Calculation:

Taking the concentration of standard solution as y axis and A as x axis create standard curve, put  $\Delta A$  into the equation and calculate the reducing sugar content y (mg/mL).

### 1. Calculated by protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 mg of reducing sugar per hour every milligram of protein.

$$\beta\text{-1,3-GA (U/mg prot)} = (y \times V1) \div (V1 \times Cpr) \div T = y \div Cpr$$

### 2. Calculated by sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 mg of reducing sugar per hour every gram of sample.

$$\beta\text{-1,3-GA (U/g fresh weight)} = (y \times V1) \div (W \times V1 \div V2) = y \div W$$

### 3. Calculated by bacteria or cell amount:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 mg of reducing sugar per hour every 10 thousand bacteria or cells.

$$\beta\text{-1,3-GA (U/10}^4 \text{ cell)} = (y \times V1) \div (500 \times V1 \div V2) = 0.002 \times y$$

V1: Sample volume, 0.035 mL;

V2: Extraction volume, 1 mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

T: The reaction time, 60 min = 1 h

500: Bacteria or cell amount, 5 million.

**Recent Product Citations:**

[1] X Niu,Q Xu,W Wang,et al. The antifungal activity of a thaumatin-like protein from oyster *Crassostrea gigas*. *Invertebrate Survival Journal*. June 2018;(IF0.967)

**References:**

[1] Mohammadi M, Karr A L. Beta-1, 3-glucanase and chitinase activities in soybean root nodules[J]. *Journal of plant physiology*, 2002, 159(3): 245.

**Related Products:**

- NA0838/NA0596  $\beta$ -1,3-glucanase( $\beta$ -1,3-GA) Activity Assay Kit
- NA0687/NA0446 Phosphoglycerate Kinase(PGK) Activity Assay Kit
- NA0686/NA0445  $\beta$ -glucosidase( $\beta$ -GC) Activity Assay Kit
- NA0685/NA0444  $\alpha$ -galactosidase( $\alpha$ -GAL) Activity Assay Kit
- NA0684/NA0443  $\beta$ -galactosidase( $\beta$ -GAL) Activity Assay Kit