β-1,3-glucanase(β-1,3-GA) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: NA0838

Size:50T/24S

Components:

Extract Solution: Liquid 50 mL×1. Storage at 4℃.

Reagent I: Powder×1. Storage at 4℃. Dissolve with 3 mL of distilled water before use.

Reagent II: Liquid 42 mL×1. Storage at 4℃.

Standard: Powder×1. Storage at 4℃. 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℃ and use within one week.

Product Description

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

β-1,3-GA hydrolyzes laminarin and inner cuts β-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, desk centrifuge, adjustable pipette, 1 mL glass cuvette, mortar, ice and distilled 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℃ 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 Extraction 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 Extraction Solution. Use ultrasonic to splitting bacteria and cell (placed on ice, 20%, work time 3s，interval 10s，repeat for 30 times). Centrifuge at 12000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before test.

II. Determination procedure:

1. Preheat the spectrophotometer 30 minutes, adjust wavelength to 540 nm, set zero with distilled water.
2. Standard preparation: Dilute the 10 mg/mL glucose standard solution to 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(μL)	Test tube	Control tube	Standard tube	Blank tube
Sample	100	100	-	-
Standard Solution	-	-	100	-
Distilled water	-	100	100	200
Reagent I	100	-	-	-
Mix thoroughly, put in 37℃ water bath for 60 minutes				
Reagent II	600	600	600	600

Mix thoroughly, boiling water bath for 5 minutes (cover tightly to prevent water loss), detect the absorbance after cooling with running water. ΔA=A(T)-A(C), A=A(S)-A(B). Each test tube shall be provided with a contrast tube.

If the absorbance is greater than 2, dilute sample with Extract solution, multiply 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 ΔA 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.

β-1,3-GA(U/mg prot) =(y×V1)÷(V1×Cpr) ÷T= y÷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.

β-1,3-GA (U/g fresh weight) =(y×V1)÷(W×V1÷V2) = y÷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.

β-1,3-GA (U/10^4 cell) =(y×V1)÷(500×V1÷V2)=0.002×y

V1: Sample volume, 0.1 mL;

V2: Extraction volume, 1 mL;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

500: Bacteria or cell amount, 10^4.

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 β-1,3-glucanase(β-1,3-GA) Activity Assay Kit
NA0687/NA0446 α-glucosidase(α-GC) Activity Assay Kit
NA0686/NA0445 β-glucosidase(β-GC) Activity Assay Kit
NA0685/NA0444 α-galactosidase(α-GAL) Activity Assay Kit