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β-1,3-glucanase(β-1,3-GA) Assay Kit

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

Operation Equipment: Microplate reader

Catalog Number: AK0555

Size:100T/48S

Components:

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

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

Reagent Ⅱ: Liquid 30 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/microplate reader, desk centrifuge, adjustable pipette, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, illed 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 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℃ 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 (μL)	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℃ 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 μL to micro glass cuvette/96 well flat-bottom plate, detect the absorbance after cooling with running water. ΔA =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 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, 0.035 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：

AK0556/AK0555	β- 1,3-glucanase(β- 1,3-GA) Activity Assay Kit
AK0209/AK0208	Phosphoglycerate Kinase(PGK) Activity Assay Kit
AK0207/AK0206	β-glucosidase(β-GC) Activity Assay Kit
AK0205/AK0204	α-galactosidase(α-GAL) Activity Assay Kit
AK0203/AK0202	β-galactosidase(β-GAL) Activity Assay Kit