Pectinase Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: NA0680

Size:50T/24S

Components:

Extract solution: 50 mL ×1. Storage at 4℃.

Reagent I: 40 mL×1, stored at 4℃, If there are insoluble substances in the solution, they can be dissolved in a water bath at 50℃.

Reagent III: 40 mL×1, stored at 4℃ and protect from light.

Standard: Powder×1, 10 mg galacturonic acid. Before use, add 0.943 mL of distilled water to prepare a standard solution of 50 μmol/mL.

Product Description:

Pectinase is one of the enzymes that decompose pectin, including protopectinase, pectinesterase, polygalacturonase and pectinase. It widely exists in fruits of higher plants and microorganisms and is the most important enzyme in fruit processing.

Pectinase hydrolyzes pectin to produce galacturonic acid, which reacts with DNS reagent to produce brownish red substance with characteristic absorption peak at 540 nm. The activity of pectinase can be calculated by measuring the change of absorption value at 540 nm.

Reagents and Equipment Required but Not Provided

Spectrophotometer, table type centrifuge, water bath, 1 mL glass cuvette, adjustable pipette, mortar/homogenizer, ice and distilled water.

Procedure

I. Extraction of crude enzyme solution:

1. Tissue sample: the proportion of tissue mass(g): volume of Extract solution (mL): 1:5~10 (it is recommended to weigh about 0.1 g of tissue, add 1 mL of Extract solution) for ice bath homogenate, then centrifuge at 10000 ×g for 10 minutes at 4℃, take the supernatant, place it on ice for testing.

2. Fungi sample: the number of cells (10^4): the volume of the Extract solution (mL) is 500-1000:1 (1 mL of the Extract solution is recommended to be added to 5 million cells), the Extract solution is added, and the cells are broken by ultrasonic wave in ice bath (Power: 300W, ultrasonic: 3s, interval: 7s, total time: 3 minutes). Centrifuge at 10000 ×g for 10 minutes at 4℃, and the supernatant is taken for test.

3. Serum sample: direct determination.

II. Test procedure

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 540 nm, and adjust to

zero with distilled water.

2. Dilute 50 μmol/mL standard solution with distilled water to 6, 5, 4, 3, 2, 1 μmol/mL standard solution for standby.

3. Take 125 μL of sample at boiling water bath for 10 min.

4. Operation table: (in 1.5 mL centrifugal tube)

Reagent name (μL)	Contrast tube (A_C)	Test Tube (A_T)	Standard tube (A_S)	Blank Tube (A_B)
Reagent I	500	500	500	500
Incubation at 50℃ water bathfor 5 minutes.				
Standard solution	-	-	125	-
Sample	-	125	-	-
Distilled water	-	-	-	125
The boiling sample	125	-	-	-
Mix well, react in water bath at 50℃ for 30 minutes, immediately boiling for 5 minutes, after cool down, centrifuge at 8000 ×g for 10 minutes at room temperature, take the supernatant.				
Supernatant	500	500	500	500
Reagent II	500	500	500	500
After boiling water bath for 5 minutes, the reaction is stopped by cooling in ice bath. Determine the absorption value a at 540 nm. The $\Delta A = A_T - A_C$ and the $\Delta A_S = A_S - A_B$ are calculated. Each testing tube shall be provided with a pair of care.				

III. Calculation of Pectinase:

1. Drawing of standard curve:

Take the concentration of each standard solution as the x-axis, and the corresponding ΔA_S as the y-axis, draw the standard curve, and get the standard equation y=kx+b, and bring ΔA into the equation to get x (μmol/mL)

2. Calculation of Pectinase

(1) Calculated by tissue protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol of galacturonic acid in the reaction system per hour at 50℃ and pH 3.5 every mg protein.

Pectinase activity (U/mg prot)=$x \times V_E \div (V_E \times Cpr) \div T = 2x \div Cpr$

(2) Calculated by the quality of tissue samples:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol of galacturonic acid in the reaction system per hour at 50℃ and pH 3.5 every g sample.

Pectinase activity (U/g fresh weight)=$x \times V_E \div W \div T = 2x \div W$

(3) By cell number:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol of galacturonic acid in the reaction system per hour at 50℃ and pH 3.5 every 10^4 cells.

Pectinase activity (U/10^4 cell) =$x \times V_E \div T \div$ number of cells (10^4) = $2x \div$number of cells (10^4)

(4) Calculated by liquid volume:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation

of 1 μmol of galacturonic acid in the reaction system per hour at 50℃ and pH 3.5 every mL liquid.
Pectinase activity (U/mL)=$x \times V_S \div V_S \div T=2x$

V_E: Volume of extract solution, 1 mL;
V_S: Volume of added sample, 0.125 mL;
Cpr: Concentration of sample protein, mg/mL;
W: Mass of sample, g;
T: Reaction time: 0.5 hour.

Note:

1. When A is greater than 1.5, it is recommended to dilute the sample before determination.
2. It is recommended to dilute the sample 10 times or 20 times before determining the fruit tissue of the plant.

Experimental example:

1. Take 0.1g kiwifruit and add 1 mL Extract solution for ice bath homogenization, then centrifugation at 4℃ and 10000g for 10min, take the supernatant and dilute 10 times, then operate according to the determination steps, measure with 96 well plate and calculate $\Delta A = A_T - A_C = 1.465 - 1.45 = 0.015$, bring in the standard curve y = 0.2575x-0.2214, calculate x = 0.918 μmol/mL, calculate the enzyme activity according to the sample mass

Pectinase activity (U/g mass) = 2x ÷ W×10 (dilution ratio) =183.6 U/g mass.

Recent Product Citations：

[1] Yuxing Wu,Liangsheng Xu,Zhiyuan Yin,et al. Transcription factor VmSeb1 is required for the growth, development, and virulence in Valsa mali. Microbial Pathogenesis. October 2018;132-138.(IF2.581)

[2] Yuxing Wu,Liangsheng Xu,Zhiyuan Yin,et al. Two members of the velvet family,VmVeA and VmVelB,affect conidiation,virulence and pectinase expression in Valsa mali. Molecular Plant Pathology. November 2017;(IF4.379)

Related Products：

NA0327 /NA0518 Protopectin Content Assay Kit
NA0329/NA0328 Soluble Pectin Content Assay Kit
NA0343/NA0342 Ionic Bound Pectin(ISP) Activity Assay Kit
NA0679/NA0437 Pectin Lyase Activity Assay Kit