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Pectinase Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ microplate reader

Cat No: AK0194

Size:100T/48S

Components:

Extract solution: 100 mL ×1. Storage at 4℃ .

Reagent I: 25 mL×1, stored at 4℃, If there are insoluble substances in the solution, they can be dissolved in a water bath at 50℃;

Reagent II: 20 mL×1, stored at 4℃;

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/microplate reader, table type centrifuge, water bath, micro glass cuvette/96 well flat-bottom plate, adjustable pipette, mortar/homogenizer, ice and distilled water.

Procedure

1. 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 0.1g of tissue, add 1 mL of Extract solution) for ice bath homogenate. Centrifuge at 10000 ×g for 10 minutes at 4℃, take the supernatant and 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℃, take the supernatant and place it on ice for testing.

3. Serum sample: direct determination.

2. Test procedure

1. Preheat the spectrophotometer/microplate reader 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 10, 8, 6, 4, 2, 1 μmol/mL standard solution for standby.

3. Take 40 μL of sample at boiling water bath for 10 minutes.

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	200	200	200	200
Incubation at 50℃ water bathfor 5 minutes.				
Standard solution	-	-	40	-
Sample	-	40	-	-
Distilled water	-	-	-	40
The boiling sample	40	-	-	-
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	150	150	150	150
Reagent II	150	150	150	150
After boiling water bath for 5 minutes, the reaction is stopped by cooling in ice bath. Take 200 μL in a micro glass cuvette/96 well flat-bottom plate to 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 (10000) = $2x \div$number of cells (10000)

(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, 0.04 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 apple and add 1ml extract for ice bath homogenization, then 10000g apple, centrifugation at 4℃ for 10min, take the supernatant and dilute 10 times, then operate according to the determination steps, measure with 96 well plate and calculate $\Delta A = A_T - A_C$ = 1.466- 1.357 = 0. 109, bring in the standard curve y = 0. 1428x-0. 1022, calculate x = 1.479 μmol/mL, calculate the enzyme activity according to the sample mass
Pectinase activity (U/g mass) = 2x ÷ W×10 (dilution ratio) = 295.8 U/g mass.

Related Products:

AK0131 /AK0130	Protopectin Content Assay Kit
AK0114/AK0113	Soluble Pectin Content Assay Kit
AK0112/AK0111	Ionic Bound Pectin(ISP) Activity Assay Kit
AK0193/AK0192	Pectin Lyase Activity Assay Kit