Ploygalacturonase (PG) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: BC2665

Size: 100T/48S

Components:

Extract solution: Liquid 60 mL×1.Store at 2-8℃.

Reagent I: Liquid 10 mL×1. Store at 2-8℃. If there is a precipitate in the solution, it can be dissolved in a water bath at 37°C.

Reagent II: Powder×1. Store at 2-8℃. Before use, add 10 mL of distilled water and put in 60℃ water bath to help dissolve.

Reagent III: Liquid 20 mL×1. Store at 2-8℃.

Standard: Powder×1. Store at 2-8℃. 10 mg galacturonic acid. Before use, add 0.943 mL of distilled water to prepare a standard solution of 50 μmol/mL.

Product Description:

Polygalacturonase (PG) is a type of pectinase that is widely found in plants, bacteria and fungi. It catalyzes the decomposition of polygalacturonic acid, and plays an important role in softening fruits, pollen pollination, maturity of seeds, and shedding of organs. When pathogenic bacteria infect host plants, they can secrete polygalacturonase to degrade the host the cell wall, in turn, leads to the development of the disease course.

PG hydrolyzes polygalacturonic acid to generate galacturonic acid, and galacturonic acid reacts with DNS reagents to produce a brown-red substance with a characteristic absorption peak at 540 nm. Pectinase activity can be calculated by measuring the change in absorbance at 540 nm.

Equipment and Reagents Required but Not Provided:

Spectrophotometer/microplate reader, centrifuge, water bath, mortar/ homogenizer/cell ultrasonic crusher, micro glass cuvette/ 96 well flat-bottom plate, transferpettor, ice and distilled water.

Procedure:

I. Sample Extraction:

1. Tissue sample: According to the mass of the tissue (g): the volume of the extract solution (mL) is 1: 5-10. It is suggested to add 1mL of extract solution to 0.1g of tissue. Fully grind on ice, centrifugate at 16000g and 4℃ for 10min. Supernatant is placed on ice for test.
2. Bacteria or cells: According to the number of cells (10^4): the volume of the extract solution (mL) is 500-1000: 1. It is suggested to add 1mL of extract solution to 5 million bacteria or cells. Use cell ultrasonic crusher to split bacteria or cells (power 300W, work time 3s，interval 7s，total time 5 min). Centrifugate at 16000g and 4℃ for 10min. Supernatant is placed on ice for test.
3. Liquid: directly measure or detect after diluting with extract solution.

II. Determination:

1 Preheat the spectrophotometer/microplate reader for 30min, adjust wavelength to 540nm and set spectrophotometer counter to zero with distilled water.

2 Standard working solution: Dilute 50 μmol/mL standard solution to 6, 5, 4, 3, 2, 1.5 μmol/mL standard solution for future use.

3 Add reagents with the following list (in a 1.5 mL centrifuge tube):

Reagent (μL)	Test tube (T)	Control tube (C)	Blank tube (B)	Standard tube (S)
sample	25	25	-	-
Distilled water	-	-	25	-
Standard solution	-	-	-	25
Reagent I	50	50	50	50
Reagent II	50	-	50	50
After accurately reacting at 40℃ for 2h, the boiling water bath is heated for 10 min (close tightly to prevent water loss), and then the EP tubes is taken out and cooled to room temperature.				-
Reagent II	-	50	-	-
Reagent III	125	125	125	125
Heat in boiling water bath for 5 min (cover tightly to prevent water loss), take it out and cool it to room temperature. After thorough mixing, absorb 200μL reaction solution and measure the absorbance at 540nm, record A_T, A_C, A_B, A_S. Calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$. A control tube is required for each test tube, and the standard curve and blank tube need only be tested once or twice.				

III. Calculation:

1. Standard curve

The concentration of standard solution as x-axis, ΔA_S as y-axis, obtain the equation y=kx+b. Take ΔA_T to the equation to acquire x (μmol/mL) value.

2. Calculation

1) Tissue protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the decomposition of 1μmol galacturonic acid every mg of protein in the reaction system per hour at 40℃ and pH6.0.

PG Activity (U/mg prot)=x×Ve÷（Ve×Cpr）÷T =0.5x÷ Cpr

2) Tissue weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the decomposition of 1μmol galacturonic acid every gram of tissue in the reaction system per hour at 40℃ and pH6.0.

PG Activity (U/g weight) = x×Ve÷ W÷T =0.5x÷ W

3) Liquid

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the decomposition of 1μmol galacturonic acid every milliliter of liquid sample in the reaction system per hour at 40℃ and pH6.0.

PG Activity (U/mL) =x× Vs÷Vs÷T=0.5x

4) Bacteria or cultured cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the decomposition of 1μmol galacturonic acid every 10^4 cells or bacteria in the reaction system per hour at 40℃ and pH6.0.

PG Activity (U/10^4 cell) =x× Ve÷ N÷ T= 0.5x÷ N

Vs: Sample volume, 0.025 mL;

Ve: Extract solution volume, 1 mL;

Cpr: Supernatant sample protein concentration, mg/mL;

T: Reaction time, 2 hours;

W: Sample weight, g;

N: Bacteria or cultured cells number, 10^4 per unit.

Note

1. The supernatant of sample extraction shall be placed on ice for testing, and it is recommended to complete the test within the same day after sample extraction.
2. If the A_T is larger than 2, it is recommended to dilute the sample with extract solution before measuring and multiply the dilution factor in the calculation formula.
3. It is suggested that the fruit samples be diluted 10 or 20 times before determination.
4. If the sample ΔA is too small, it is recommended to extend the enzymatic reaction time and divide it by the corresponding time in the calculation formula.

Experimental example:

1. Take 0.1g of hibiscus flower and add 1 mL extract solution ice bath to homogenate, and then centrifuge at 4℃ and 16000g for 10 min, and dilute it for 2 times, and then operate according to the measurement procedure. Calculate $\Delta A = A_T - A_C = 1.6843 - 1.4916 = 0.1927$, and bring the standard curve y= 0.2426x-0.2979, and calculate x = 2.022 μmol/mL, and calculate according to the sample weight:

PG activity (U/g weight) = 0.5x ÷ W × 2 (dilution ratio) =20.22 U/g weight.

Related Products:

BC2630/BC2635	Pectinase Activity Assay Kit
BC3680/BC3685	Protopectin Content Assay Kit
BC4150/BC4155	Ionic Bound Pectin(ISP) Activity Assay Kit
BC2640/BC2645	Pectin Lyase (PL) Activity Assay Kit