Ploygalacturonase (PG) Activity Assay Kit

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

Detection instrument: Spectrophotometer

Cat No: BC2660

Size: 50T/24S

Components:

Extract solution: 30 mL×1.Store at 4℃.

Reagent I: 8 mL×1. Store at 4℃. 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 4℃. Before use, add 8 mL of distilled water and put in 60℃ water bath to help dissolve.

Reagent III: 20 mL×1.Store at 4℃

Standard: Powder×1. Store at 4℃. 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:

Desk centrifuge, spectrophotometer, constant temperature water bath, mortar/homogenizer, 1 mL glass cuvette, 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. Suggested 0.1g of tissue with 1mL of extract solution. 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. Suggest 5 million with 1mL of Extract Solution. Use ultrasonication to split bacteria or cells (power 300W, work

time 3s，interval 7s，total time 3 min). centrifugated at 16000g and 4℃ for 10min. Supernatant is placed on ice for test.

3. Liquid: direct measurement.

Determination procedure:

1 Preheat the spectrophotometer 30min, adjust wavelength to 540nm, set zero with distilled water.

2 Dilute 50 μmol/mL standard solution to 10, 6, 4, 3, 2, 1.5, 1.2 μmol/mL standard solution for future use.

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

Reagent name (μL)	Test tube (T)	Control tube (C)	Blank tube (B)	Standard tube (S)
sample	50	50		
Distilled water	-	-	50	
Standard solution	-			50
Reagent I	100	100	100	100
Reagent II	100		100	100
After accurately reacting at 40℃for 2 h, 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		100		
Reagent III	250	250	250	250
Heat in boiling water bath for 5 min (cover tightly to prevent water loss), take it out and cool it to room temperature.				
Distilled water	500	500	500	500

After thorough mixing, measure the absorbance A at 540nm, record A_T, A_C, A_B, A_S. Calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$.

II. 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℃.

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℃.

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℃.

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℃.

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

Vs: Sample volume (mL), 0.05 mL;

Ve: Extract solution volume, 1 mL;

Cpr: Supernatant sample protein concentration (mg/mL);

T: Reaction time (min), 2 hours;

W: Sample weight, g;

Cell amount: 10 thousand as 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 1.2, it is recommended to dilute the sample with extract solution before measuring.
3. It is suggested that the 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 5 times, and then operate according to the measurement procedure. Calculate $\Delta A = A_T - A_C = 0.912 - 0.882 = 0.03$, and bring the standard curve y=0.1127x-0.1207, and calculate x = 1.337 μmol/mL, and calculate according to the sample quality:

PG enzyme activity (U/g mass) = 0.5x ÷ W × 2 (dilution ratio) =33.43 U/g mass.

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 Activity Assay Kit