Pectin lyase (PL) Activity Assay Kit

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

Detection instrument: Spectrophotometer/ Microplate reader

Cat No: BC2645

Size: 100T/96S

Components:

Extract solution: Liquid 120 mL×1.Store at 2-8℃.

Reagent I: Liquid 15 mL×1. Store at 2-8℃. If there is a precipitate in the solution, it can be dissolved in a water bath at 50°C.

Product Description:

Pectin lyase (EC4.2.2.10) is an important part of pectinase and catalyzes the elimination and cleavage of pectin molecular chain. The sources are relatively wide, mainly derived from microorganisms, which is of great significance in increasing fruit juice production in the food processing industry, and also has potential application value in reducing environmental pollution and energy consumption.

Pectin lyase acts on the α-1,4 glycosidic bond in pectin, and generates unsaturated oligogalacturonic acid having an unsaturated bond between the reducing end C4 and C5. It has a characteristic absorption peak at 235nm. The increase in absorbance at 235 nm is measured to indicate the activity of pectin lyase.

Required material

Spectrophotometer/microplate reader, centrifuge, constant temperature water bath, transferpettor, micro quartz cuvette/96 well UV plate, mortar/homogenizer/cell ultrasonic crusher, 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 0.1 g of tissue with 1 mL of Extract solution. Fully grind on ice, centrifuge at 10000g and 4℃ for 10 min. 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 1 mL of Extract Solution. Use ultrasonication to split bacteria or cells (power 300W, work time 3s，interval 7s，total time 3 min). Centrifuge at 10000g and 4℃ for 10 min. Supernatant is placed on ice for test.

3. Culture medium: direct measurement.

Determination procedure:

1 Preheat the spectrophotometer/microplate reader 30 min, adjust wavelength to 235 nm, and set spectrophotometer counter to zero with distilled water.

2 Add reagents with the following list:

Reagent (μL)	Test tube (T)	Blank tube (B)

Reagent I	180	180
Enzyme solution	20	-
Distilled water	-	20

After thorough mixing, measure the initial value A1 at 235 nm, measure the absorbance A2 after reacting at 40°C for 30 minutes, and calculate $\Delta A_T = A2_T - A1_T$, $\Delta A_B = A2_B - A1_B$, $\Delta A = \Delta A_T - \Delta A_B$.

II. Calculation:

1 Calculated by micro quartz cuvette

1) Calculated by protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1nmol of unsaturated galacturonic acid in the reaction system per minute at pH=5.5 and 40°C every mg protein.

PL activity (U/mg prot) $=\Delta A \div (\varepsilon \times d) \times V_{RV} \times 10^9 \div (V_S \times Cpr) \div T = 64.1 \times \Delta A \div Cpr$

2) Calculated by sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1nmol of unsaturated galacturonic acid in the reaction system per minute at pH=5.5 and 40°C every g sample.

PL activity (U/g weight) $=\Delta A \div (\varepsilon \times d) \times V_{RV} \times 10^9 \div (V_S \times W \div V_{ST}) \div T = 64.1 \times \Delta A \div W$

3) Calculated by bacteria or cell amount:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1nmol of unsaturated galacturonic acid in the reaction system per minute at pH=5.5 and 40°C every 10^4 bacteria or cells.

PL activity (U/10^4 cell) $=\Delta A \div (\varepsilon \times d) \times V_{RV} \times 10^9 \div (V_S \times N \div V_{ST}) \div T = 64.1 \times \Delta A \div N$

4) Calculated by volume of culture medium:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1nmol of unsaturated galacturonic acid in the reaction system per minute at pH=5.5 and 40°C every mL liquid.

PL activity (U/mL) $=\Delta A \div (\varepsilon \times d) \times V_{RV} \times 10^9 \div V_S \div T = 64.1 \times \Delta A$

ε: molar extinction coefficient of unsaturated galacturonic acid: 5200 L/mol/cm;

d: light path of cuvette, 1 cm;

V_{RV}: total reaction volume, 0.0002 L;

V_S: sample volume in reaction system, 0.02 mL;

V_{ST}: volume of extraction solution added, 1 mL;

Cpr: sample protein concentration, mg/mL;

W, sample mass, g;

T: reaction time, 30 min;

10^9: conversion factor, 1 mol = 10^9 nmol;

N: Number of bacteria or cells.

2. Calculated by 96 well UV plate:

Modify d-1 cm in the above formula to d-0.6 cm (light path of 96 well UV plate) for calculation.

Note

1. If ΔA is greater than 0.5, dilute the crude enzyme solution with distilled water before measuring. If the A_T is larger than 1.5, it is recommended to dilute the sample with distilled water before measuring.
2. It is recommended not to measure too many samples at one time to avoid delaying too much enzymatic reaction time.
3. Normally, the blank tube does not change more than 0.02.

Experimental example:

1. Take 0.1g of banana peel and add 1 mL of Extract solution to homogenize in ice bath. After centrifugation at 4℃ and 10000g and for 10 min, the supernatant is taken out and put on ice for determination. Then, according to the determination steps, calculate ΔA with a micro quartz cuvette, $\Delta A_T=A2_T-A1_T= 0.528-0.5254 = 0.0025$, and calculate the enzyme activity according to the sample mass.

PL activity (U/g weight) = 64.1 × ΔA ÷ W = 1.5964 U/g weight.

2. Take an appropriate amount of Escherichia coli (5 million) and add 1 mL of Extract solution to ice bath ultrasonic to crush cells (power 300W, ultrasonic 3s, interval 7s, total time 3 min); then centrifuge at 10000g and 4℃ for 10min, take the supernatant and put it on ice, then operate according to the determination steps, measure with micro quartz cuvette and calculate the $\Delta A_T = A2_T- A1_T= 1.2213-0.8277 = 0.3936$, and calculate the enzyme activity according to bacteria amount.

PL activity (U/10^4 cell) = 64.1 × ΔA ÷ N = 241.67 U/10^4 cell.

Related Products：

BC2630/BC2635	Pectinase Activity Assay Kit
BC3680/BC3685	Protopectin Content Assay Kit
BC4150/BC4155	Ionic Bound Pectin(ISP) Activity Assay Kit
BC2660/BC2665	Ploygalacturonase(PG) Activity Assay Kit