

Pectinesterase (PE) Activity Assay Kit

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

Cat No: BC2700

Size: 100T/96S

Components:

Extract solution: Powder×2, store at room temperature. Add 100 mL distilled water to each bottle before use. It could be stored at 2-8°C for three months.

Reagent I: Powder×1, store at room temperature. Add 100 mL distilled water, heat at 50°C for 3 hours to dissolve completely, then transfer it to a 500 mL volumetric flask after cooling, and use distilled water to make the volume constant. It could be stored at 2-8°C for eight weeks.

Reagent II: Liquid 3mL×1, store at 2-8°C.

Reagent III: Liquid 20mL×1, store at 2-8°C.

Reagent III working solution: Dilute Reagent III 16 times with distilled water according to sample number. It could be stored at 2-8°C for one week.

Description:

Pectinesterase is widely found in plants and microbes. Pectin is one of the main components of plant cell wall. Pectinesterases catalyze the de-esterification of pectin into pectate and methanol, which play important roles in cell wall metabolism during fruit ripening.

It produces H⁺ in the process of pectin hydrolysis catalyzed by pectinesterase, and pH value of the solution decreases. The pH value of the solution could be adjusted to 7.8 by adding alkaline solution, and the activity of pectinesterase could be calculated by consumed volume of alkaline solution.

Required but not provided:

Centrifuge, constant temperature foster box/water-bath, transferpettor, mortar/homogenizer, 10mL tube, volumetric flask, ice and distilled water.

Protocol:

I. Sample Preparation.

1. Precool mortar/homogenizer and Extract solution on ice or at 4°C for 10min.
2. The weight (g): volume of Extract solution (mL)= 1:3 - 5, Suggest that weigh 0.5 g of sample, add 1.5 mL of Extract solution and homogenate in ice bath. Centrifuge at 4°C and 12000g for 10 minutes and discard precipitation, take the supernatant on ice for test

II. Determination Procedure:

1. Preheat Reagent I at 37°C for 10min.
2. Add 1mL supernatant of samples, 25μL Reagent II and 4mL Reagent I into 10mL tube. Mix well. Adjust mixture pH to 7.8 (pink) with Reagent III working solution.
3. Incubate for 60 minutes at 37°C constant temperature foster box/water-bath, adjust pH to 7.8

(pink) with Reagent III working solution every 20 minutes and record volume of added Reagent III working solution as V(mL).

III. PE Activity Calculation

Unit definition: One unit of enzyme activity is defined as that per gram of sample consumes 1 μmol of NaOH per min.

$$\text{PE activity (U/g weight)} = 25 \times V \div V_S \times V_E \div W \div T \times F = 1.25 \times V \times F$$

V: Consumed volume of Reagent III working solution, mL;

V_S: Sample supernatant volume, 1 mL;

V_E: Add the volume of Extract solution, 1.5 mL;

W: Sample weight, g;

T: Reaction time, 60min;

F: Dilution times.

Note:

1. Enzymatic reaction produces H⁺, and the solution is colorless. The solution suddenly changes from colorless to pink when its pH is adjusted to 7.8 with Reagent III working solution.
2. It is necessary to pre-experiment before formal determination. During the determination procedure 2, the volume of added Reagent III working solution could be adjusted to prevent the pH value is too high.
3. If samples enzyme activity is too high, it is suggested that the samples should be diluted with multiple times of the Extract solution. Sample supernatant volume could be increased if samples enzyme activity is too low. And modify the calculation formula.

Experimental example:

1. Take 0.51g apple, add 1.5 ml Extract solution, grind the homogenate with ice bath, centrifuge at 4°C and 8000g for 10min, and place the supernatant on ice. Then operate according to the determination steps, the mixture is incubated for 60 minutes at 37°C, 0.76mL Reagent III working solution is consumed to adjust pH, and calculate the enzyme activity according to the sample mass:

$$\text{PE activity (U/g weight)} = 25 \times V \div V_S \times V_E \div W \div T = 0.93 \text{ U/g weight.}$$

Related Products:

BC2540/BC2545	Cellulase (CL) Assay Kit
BC2630/BC2635	Pectinase Activity Assay Kit
BC2640/BC2645	Pectin Lyase Activity Assay Kit
BC4290/BC4295	N-Acetyl- β -D-Glucosidase (NAG) Activity Assay Kit
BC4440/BC4445	Hemicellulose Content Assay Kit