

Water-Soluble Pectin (WSP) Content Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC4120

Size: 50T/24S

Components:

Extract solution I: 100 mL of 80% ethanol. Take 80 mL of ethanol and add 20 mL of distilled water, **self-provided reagent**.

Extract solution II: 30 mL×1, stored at 4°C.

Extract solution III: 70 mL×1, stored at 4°C.

Reagent I: 60 mL of concentrated H₂SO₄, self-prepared.

Reagent II: 3 mL×1, stored at 4°C.

Reagent III: 5 mL×1, stored at 4°C.

Standard: Powder×1, 10 mg of galacturonic acid, stored at 4°C. Before use, add 0.943 mL extract solution III to prepare a standard solution of 50 μmol/mL.

Product Description

Pectin is the main component of primary cell wall and mesosol, which softens and binds cells. The pectin are crosslinked by Ca²⁺ bridge and other ion bonds, hydrogen bonds, glycoside bonds, ester bonds and benzene ring coupling. Various pectin can be extracted by different extraction methods, such as water-soluble pectin (WSP), ion-bound pectin (ISP) and covalently bound pectin (CSP).

The water-soluble pectin is hydrolyzed to galacturonic acid in acid condition, and the latter condensed with carbazole in sulfuric acid solution to form a purplish red compound. The product has the maximum absorption peak at 530 nm

Reagents and Equipment Required but Not Provided.

Spectrophotometer, low temperature centrifuge, water bath, 1 mL glass cuvette, adjustable pipette, mortar/homogenizer, **acetone, concentrated H₂SO₄, anhydrous ethanol** and distilled water.

Procedure

I. Extraction of protopectin

Take about 0.1 g of sample, add 1 mL of extract solution I, rapidly homogenization at room temperature, water bath at 95°C for 20 minutes, cool to room temperature. Centrifuge at 4000 ×g for 10 minutes at 25°C, discard the supernatant. Add 1.5 mL of extract solution I and acetone to the precipitate and wash them twice alternately (vortex oscillation for 2 minutes, centrifuge at 4000×g for 10 minutes at 25°C, discard supernatant). The precipitate is the rough cell wall. Add 1 mL of extract II (starch removal) to soak for 15 hours. Centrifuge at 4000 ×g for 10 minutes at 25°C, discard the supernatant, add 1 mL of extract

solution III, and fully homogenize. Centrifuge at $8000 \times g$ for 10 minutes at 25°C and take the supernatant for test.

II. Measurement steps:

- Preheat the spectrophotometer for 30 minutes, adjust the wavelength to 530 nm and adjust zero with distilled water.
- Dilute 50 $\mu\text{mol/mL}$ standard solution to 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 $\mu\text{mol/mL}$ standard solution for standby.
- Operation table:

Reagent name	Blank tube (B)	Standard tube (S)	Contrast tube (C)	Test tube (T)
Sample (μL)	-	-	100	100
Standard (μL)	-	100	-	-
Distilled water (μL)	100	-	-	-
Reagent I (μL)	800	800	800	800
Mix well, place at 90°C for 10 minutes, take out and cool down				
Reagent II (μL)	-	-	100	-
Reagent III (μL)	100	100	-	100
Mix well, let it stand at 25°C for 30 minutes, and measure the absorbance value at 530 nm, and record it as A_B , A_S , A_C and A_T respectively. $\Delta A_S = A_S - A_B$, $\Delta A_T = A_T - A_C$.				

III. Calculation of Betaine Content:

1. Drawing of standard curve:

Take ΔA_S as y-axis, standard solution concentration as x-axis, draw standard curve, get standard equation $y = kx + b$, bring ΔA_T into the equation, get x (mg/mL).

2. Calculation of protopectin content:

protopectin content ($\mu\text{mol/g}$ Fresh weight) $= x \times V_{\text{EIII}} \div W = 2x \div W$.

V_{EIII} : volume of extract solution III, 2 mL;

W: Fresh weight of sample, g.

Note:

- Concentrated H_2SO_4 is highly corrosive, so special attention shall be paid during operation. After heating at 90°C , take it out, cool it and then open the cover to prevent liquid splashing and burning.
- If ΔA is more than 0.5, the sample can be appropriately diluted with extract solution III and then determined, and multiplied by the dilution multiple in the calculation formula.

Experimental Examples:

1. Take 0.1g of poplar leaves and add 1mL of extraction solution one to sample processing. Dilute the supernatant by 5 times and follow the measurement procedure to calculate $\Delta A_T = A_T - A_C = 0.079 - 0.031 = 0.048$, Bring in the standard curve $y = 0.7536x + 0.0022$ $x = 0.0608$, and calculate:

Water-Soluble Pectin content ($\mu\text{mol/g}$ mass) $= 2x \div W \times 5 = 6.08 \mu\text{mol/g}$ mass.

Related Products:

BC1400/BC1405 Pectin Content Assay Kit
BC2630/BC2635 Pectinase Activity Assay Kit