# Water-Soluble Pectin (WSP) Content Assay Kit

**Note:** Take two or three different samples for prediction before test.
**Operation Equipment:** Spectrophotometer/microplate reader
**Catalog Number:** BC4125
**Size:**100T/48S

**Components:**
Extract solution I: 125 mL of 80% ethanol. Take 100 mL of ethanol and add 25mL of distilled water, self-provided reagent.
Extract solution II: 50 mL×1, stored at 4℃.
Extract solution III: 120 mL×1, stored at 4℃.
Reagent I: 25 mL of concentrated $H_2SO_4$, self-prepared.
Reagent II: 2.5 mL×1, stored at 4℃.
Reagent III: 5 mL×1, stored at 4℃.
Standard: Powder×1, 10 mg of galacturonic acid, stored at 4℃. Before use, add 0.943 mL of 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 $Ca^{2+}$ 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/microplate reader, desktop low temperature centrifuge, water bath, micro glass cuvette/96 well flat-bottom plate, adjustable pipette, mortar/homogenizer, acetone, concentrated $H_2SO_4$, 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℃ for 20 minutes, cool to room temperature. Centrifuge at 4000 ×g for 10 minutes at 25℃, 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℃, 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℃, discard the supernatant, add 1 mL of extract solution III, and fully homogenize. Centrifuge at 8000 ×g for 10 minutes at 25℃ and take the supernatant for test.

**II. Measurement steps:**

a. Preheat the spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 530 nm and adjust zero with distilled water.

b. Dilute 50 μmol/mL standard solution to3、2、1、0.5、0.25、0.125、0.0625 μmol/mL standard solution for standby.

c. Operation table:

| Reagent name (μL) | Blank tube (B) | Standard tube (S) | Contrast tube (C) | Test tube(T) |
|---|---|---|---|---|
| Sample | - | - | 25 | 25 |
| Standard | - | 25 | - | - |
| Distilled water | 25 | - | - | - |
| Reagent I | 200 | 200 | 200 | 200 |
| Mix well, place at 90 ℃ for 10 minutes, take out and cool down. | | | | |
| Reagent II | - | - | 25 | - |
| Reagent III | 25 | 25 | - | 25 |
| Mix well, let it stand at 25℃ 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 $\Delta A_S$ as y-axis, standard solution concentration as x-axis, draw standard curve, get standard equation y = kx+b, bring $\Delta A_T$ into the equation, get x (mg/mL).

2. Calculation of protopectin content:

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

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

W: Fresh weight of sample, g.

**Note:**

1. Concentrated $H_2SO_4$ is highly corrosive, so special attention shall be paid during operation. After heating at 90℃, take it out, cool it and then open the cover to prevent liquid splashing and burning.
2. If ΔA is more than 1, 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 ,measure by the 96 well plate and calculate ΔAt=At-Ac==0.087-0.054=0.033, Bring in the standard curve y=0.431x-0.0256 x=0.136, and calculate:

Water-Soluble Pectin content (μmol/g mass) = 2x÷W×5=13.6 μmol/g mass.

**Related Products:**

BC1400/BC1405 Pectin Content Assay Kit

BC2630/BC2635 Pectinase Activity Assay Kit