

## Ion-Bonded Pectin (ISP) Content Assay Kit

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

**Operation Equipment:** Spectrophotometer

**Catalog Number:** AK0112

**Size:**50T/24S

### Components:

Extract solution I: 80% ethanol, [self-provided reagent](#), which consists of 80 mL of absolute ethanol and 20 mL of distilled water.

Extract solution II : Liquid 30 mL×1. Storage at 4°C .

Extract solution III : Liquid 50 mL×1. Storage at 4°C .

Solution I: Concentrated sulfuric acid 60 mL×1, [self-provided reagent](#).

Solution II : Liquid 3 mL×1. Storage at 4°C .

Solution III: Liquid 5 mL×1. Storage at 4°C .

Standard: Powder×1. 10 mg of galacturonic acid. Storage at 4°C . Add 0.943 mL of Extract solution III to prepare 50 μmol/mL standard solution before use.

### Product Description

Pectin is the main component of primary cell wall and mesosol, which softens and binds cells. The pectin are crosslinked by Ca<sup>2+</sup> 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).

ISP is extracted by an acid extract with chelating agent, and hydrolyzed to galacturonic acid in acid condition. Galacturonic acid condensed with carbazole in sulfuric acid solution to form a purplish red compound, which has the maximum absorption peak at 530 nm.

### Reagents and Equipment Required but Not Provided.

Centrifuge, water-bath, transferpettor, spectrophotometer, 1 mL glass cuvette, ice, methylbenzene, acetone, concentrated sulfuric acid, anhydrous ethanol and distilled water.

### Procedure:

#### I. Complex extraction:

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, centrifugate at 4000 g for 10 minutes at 25°C, discard the supernatant. Add 1.5 mL of extract I and acetone to the precipitate and wash them twice alternately (vortex oscillation for 2 minutes, centrifugate at 4000 g for 10 minutes at 25°C, discard supernatant). The precipitate is the rough cell wall. Add 1 mL of Extract solution II (starch removal) to soak for 15 hours, centrifugate at 4000 g for 10 minutes at 25°C, discard the supernatant, add 1 mL of

Extract solution III, and fully homogenize. centrifugate at 8000 g for 10 minutes at 25°C and take the supernatant for test.

## II. Determination procedure:

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 530 nm, set zero with distilled water.
2. Dilute the 50 μmol/mL standard solution to 2、1、0.8、0.6、0.4、0.2、0.1 μmol/mL standard solution with the Extract solution III for standby.
3. Add reagents with the following list:

Reagent	Blank Tube (B)	Standard Tube (S)	Contrast Tube (C)	Test Tube (T)
Sample (μL)	-	-	100	100
Standard (μL)	-	100	-	-
Distilled water (μL)	100	-	-	-
Solution I (μL)	800	800	800	800
Mix thoroughly and incubate the reaction for 10 minutes at 90°C water bath (tightly close to prevent moisture loss), flowing water to cool.				
Solution II (μL)	-	-	100	-
Solution III (μL)	100	100	-	100
Mix well, let it stand at 25°C for 30 minutes, measure the absorbance value at 530 nm, record it as A <sub>B</sub> , A <sub>S</sub> , A <sub>C</sub> and A <sub>T</sub> respectively. $\Delta A_S = A_S - A_B$ , $\Delta A_T = A_T - A_C$ .				

## III. Calculate:

### 1. 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 (μmol/mL).

### 2. Calculation

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

$V_{EIII}$ : volume of Extract solution III, 1 mL;

W: Fresh weight of sample, g.

### Note:

1. As concentrated sulfuric acid is highly corrosive, so special attention shall be paid during operation. After heating at 90°C, take it out, flowing water or ice water bath to cool. Do not open the cover until it is cooled, to prevent liquid splashing and burning.
2. If  $\Delta A$  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 Winter Jasmine leaves and add 1mL of extract 1. After sample processing, take the supernatant and operate according to the measurement procedure. Calculate  $\Delta A_t = A_t - A_b = 0.172 - 0.097 = 0.075$ , bring it into the standard The curve  $y = 0.5396x + 0.0101$ ,  $x = 0.1202$ , which is calculated by the formula:



Ion-Bonded Pectin (ISP) Content ( $\mu\text{mol/g weight}$ ) =  $x \div W = 1.202 \mu\text{mol/g weight}$ .

**Related Products:**

AK0313/AK0312 Pectin Content Assay Kit

AK0195/AK0194 Pectinase Activity Assay Kit

AK0189/AK0188 Ploygalacturonase(PG) Activity Assay Kit