Ion-Bonded Pectin (ISP) Content Assay Kit

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

Operation Equipment: Spectrophotometer Microplate Reader

Catalog Number: BC4155

Size:100T/48S

Components:

Extract solution I: 80% ethanol, self-provided reagemt, which consists of 80 mL of absolute ethanol and 20 mL of distilled water.

Extract solution II: Liquid 50 mL×1. Storage at 4℃.

Extract solution III: Liquid 70 mL×1. Storage at 4℃.

Solution I: Concentrated sulfuric acid 30 mL×1, self-provided reagemt.

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

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

Standard: Powder×1. 10 mg of galacturonic acid. Storage at 4℃. 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 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).

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/microplate reader, micro glass cuvette/96 well flat-bottom plate, 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℃ for 20 minutes, cool to room temperature, centrifugate at 4000g for 10 minutes at 25℃, 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℃, 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℃, discard the supernatant, add 1 mL of Extract solution III, and fully homogenize. centrifugate at 8000×g for 10 minutes at 25℃ and take the supernatant for test.

II. Determination procedure:

1. Preheat the spectrophotometer or microplate reader 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、0.1μmol/mL 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)	-	-	25	25
Standard (μL)	-	25	-	-
Distilled water (μL)	25	-	-	-
Solution I (μL)	200	200	200	200
Mix thoroughly and incubate the reaction for 10 minutes at 90℃ water bath (tightly close to prevent moisture loss), flowing water to cool.				
Solution II (μL)	-	-	25	-
Solution III (μL)	25	25	-	25
Mix well, let it stand at 25℃ for 30 minutes, measure the absorbance value at 530 nm, 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. Calculate:

1. 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 (μmol/mL).

2. Calculation

ISP content (μmol/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℃, 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 ΔA is more than 0.4, 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. Measure by the 96 well plate and calculate ΔAt = At-Ab =0.13-0.079=0.051, bring it into the standard The curve y=0.3693x-0.0189, x=0.189, which is calculated by the formula:

Ion-Bonded Pectin (ISP) Content（μmol/g weight）=x÷W=1.89 μmol/g weight。

Related Products:

BC1400/BC1405 Pectin Content Assay Kit

BC2630/BC2635 Pectinase Activity Assay Kit

BC2660/BC2665 Ploygalacturonase(PG) Activity Assay Kit