Covalently Bound Pectin Content Assay Kit - Spectrophotometry

Product code: 112090

Size: 50T

Product Introduction

Pectin is a major component of the primary cell wall and middle lamella. It mainly consists of protopectin, pectic acid methyl ester, and pectic acid. Pectin contains galacturonic acid, galactose, arabinose, glucuronic acid, and other components, and is one of the most abundant polysaccharides in many higher plant cell walls.

Pectin affects the texture and quality of plant-derived foods through its physical and chemical properties. Pectins are cross-linked by Ca^{2+}bridges, ionic bonds, hydrogen bonds, glycosidic bonds, ester bonds, and benzene-ring coupling.

Different extraction methods can be used to obtain water-soluble pectin (WSP), ionically bound pectin (ISP), and covalently bound pectin (CSP). CSP is extracted with an alkaline solution containing a chelating agent, and its content is determined by the carbazole colorimetric method.

In this assay, pectin is hydrolyzed into galacturonic acid, which reacts with carbazole reagent in sulfuric acid solution. The resulting product has a maximum absorption peak at 530 nm.

Reference performance data: Sample: apple peel. OD_{530nm}: blank 0.052; standard 1.488; control 0.039/0.041; assay 2.225/2.230. Actual readings may vary depending on the detection instrument and conditions.

Package Contents and Storage

Code	Component	Quantity	Storage
112090.1	Reagent I	60 mL	2-8°C
112090.2	Reagent II	60 mL	2-8°C
112090.3	Reagent III	6 mL	2-8°C
112090.4	Standard	5 mg	2-8°C
112090.m	Instructions	1 copy	/

Quality and Safety Information

Raw Material or Packaging Name	Quality Standard	Primary Toxicity
Reagent I	—	—
Reagent II	—	—
Reagent III	—	—
Standard	—	—

Transportation and Storage

Transportation: Transport this product with ice packs.

Storage: Store according to the package contents table. Shelf life: 180 days.

Instructions for Use

1. Sample Pretreatment

1.1 Extraction of Cell Walls

1. Take approximately 0.3 g of sample.
2. Add 1 mL of 80% ethanol and rapidly homogenize at room temperature.
3. Incubate in a 95°C water bath for 20 min, then cool to room temperature.
4. Centrifuge at 4000 × g, 25°C for 10 min, and discard the supernatant.
5. Add 1.5 mL of 80% ethanol to the precipitate, vortex for about 2 min, centrifuge at 4000 × g, 25°C for 10 min, and discard the supernatant.
6. Add 1.5 mL of acetone to the precipitate, vortex for about 2 min, centrifuge at 4000 × g, 25°C for 10 min, and discard the supernatant.
7. The precipitate is the crude cell wall. Add 1 mL of Reagent I to remove starch and soak for 15 hours.
8. Centrifuge at 4000 × g, 25°C for 10 min, discard the supernatant, dry the precipitate, and weigh to obtain cell wall material (CWM).

1.2 Extraction of CSP

1. Weigh 3 mg of dried CWM.
2. Add 1 mL of Reagent II and homogenize thoroughly.
3. If the dried material is hard, grind it before adding 1 mL of Reagent II, or homogenize with a homogenizer.
4. Centrifuge at 8000 × g, 4°C for 10 min.
5. Collect the supernatant for testing.

2. Reagent Preparation

Standard: The standard contains 5 mg galacturonic acid. Before use, add 1 mL distilled water to dissolve it to a 5 mg/mL standard solution. Then dilute the 5 mg/mL standard solution with distilled water to 0.2 mg/mL for use: 40 μL of 5 mg/mL standard solution + 960 μL distilled water. Store at 2-8°C.

3. Assay Procedure

1. Preheat the spectrophotometer for at least 30 min.
2. Set the wavelength to 530 nm and zero the instrument with distilled water.
3. Prepare the reaction tubes according to the table below.

Component	Blank Tube	Standard Tube	Control Tube	Assay Tube
Sample	-	-	100 μL	100 μL
Standard solution	-	100 μL	-	-
Distilled water	100 μL	-	-	-
Anhydrous ethanol	-	-	100 μL	-
Reagent III	100 μL	100 μL	-	100 μL
Concentrated sulfuric acid	800 μL	800 μL	800 μL	800 μL

1. Mix thoroughly after adding Reagent III.
2. Add concentrated sulfuric acid and mix thoroughly.
3. Incubate in a 95°C water bath for 5 min.
4. Cool to room temperature.
5. Read at 530 nm and record the absorbance values as A_{blank}, $A_{standard}$, $A_{control}$, and A_{assay}.

Calculate $\Delta A = A_{assay} - A_{control}$.

Calculate $\Delta A_{standard} = A_{standard} - A_{blank}$.

If ΔA is greater than 2.5, dilute the test sample with distilled water. The sample may be diluted 10 times or 20 times.

Only one blank tube and one standard tube are required. Each assay tube must have one corresponding control tube.

4. CSP Content Calculation

CSP content (mg/g dry weight) = ($C_{standard}$× V_{sample}) × ΔA ÷ $\Delta A_{standard}$÷ (W × V_{sample}÷ $V_{extraction}$) × dilution factor

Simplified formula:

CSP content (mg/g dry weight) = 0.2 × ΔA ÷ $\Delta A_{standard}$÷ W × dilution factor

Symbol	Meaning	Value
$C_{standard}$	Standard tube concentration	0.2 mg/mL
V_1	Sample volume added	0.1 mL
V_2	Extraction solution volume added	1 mL
W	Sample dry weight	g

Precautions

1. Before the formal assay, select 2-3 samples with large expected differences for preliminary testing. This 50T kit can test 24 samples.
2. Required instruments and supplies not provided: benchtop centrifuge, visible spectrophotometer, water bath or metal bath, adjustable pipettes, 1 mL glass cuvette, absolute ethanol, acetone, concentrated sulfuric acid, mortar or homogenizer, and distilled water.
3. The linear range of this kit is 0.003125-0.4 mg/mL.
4. If Reagent I has solidified, let it stand at room temperature until it redissolves.
5. If the measured absorbance value exceeds the absorbance value of the linear range, reduce the sample amount or dilute the sample before measurement.