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CheKine™ Micro Ionic Bound Pectin (ISP) Content Assay Kit

Cat #: KTB3016 Size: 48 T/48 S 96 T/96 S

	Micro Ionic Bound Pectin (ISP) Content Assay Kit		
REF	**Cat #:** KTB3016	LOT	**Lot #:** Refer to product label
	Detection range: 0.5-5 mg/mL		**Sensitivity:** 0.5 mg/mL
	Applicable sample: Plant Tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Pectin is a major component of the primary cell wall and middle lamella, primarily consisting of protopectin, pectic acid methylester, and pectic acid. Pectin contains galacturonic acid, galactose, arabinose, glucuronic acid, and others, making it one of the most abundant polysaccharides in the cell walls of many higher plants. Its unique physical and chemical properties affect the texture and quality of plant-derived foods. Pectins are cross-linked by Ca^{2+} bridges and other ionic bonds, hydrogen bonds, glycosidic bonds, ester bonds, and phenolic ring couplings. Various forms of pectin can be extracted through different extraction methods, such as water-soluble pectin (WSP), Ionic bound pectin (ISP), and covalently-bound pectin (CSP). CheKine™ Micro Ionic Bound Pectin (ISP) Content Assay Kit provides a simple, convenient, and rapid method for ISP content determination suitable for plant tissue samples. The principle is to extract ionic bound pectin (ISP) using an acidic solution containing chelating agents, and determine the pectin content using the carbazole colorimetric method. Pectin is hydrolyzed into galacturonic acid, which undergoes a condensation reaction with the carbazole reagent in sulfuric acid solution. The resulting substance exhibits a maximum absorption peak at 530 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	60 mL	120 mL	4℃, protected from light
Reagent Ⅱ	60 mL	120 mL	4℃
Reagent Ⅲ	4 mL	8 mL	4℃
Standard	1 mL	1 mL	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 530 nm

- Thermostatic water bath, analytical balance, ice maker, freezing centrifuge
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water, 80% ethanol, acetone, concentrated sulfuric acid
- Dounce homogenizer

Reagent Preparation

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Note: Reagent I and Reagent III are toxic and have an irritating odor, so it is recommended to experiment in a fume hood.

Standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Extract the cell wall: Weigh approximately 0.3 g of the sample, add 1 mL of 80% ethanol, and homogenize quickly at room temperature. Incubate in a 95℃ water bath for 20 min, then cool to room temperature. Centrifuge at 4,000 g at 25℃ for 10 min and discard the supernatant. Wash the pellet once with 1.5 mL of 80% ethanol and once with acetone (vortex for about 2 min, centrifuge at 4,000 g at 25℃ for 10 min, and discard the supernatant). The resulting pellet is the crude cell wall. Add 1 mL of Reagent I (to remove starch) and soak for 15 h. Centrifuge at 4,000 g at 25℃ for 10 min, discard the supernatant, dry the pellet, and weigh to obtain the cell wall material (CWM).
2. ISP Extraction: Weigh 3 mg of dried CWM and add 1 mL of Reagent II. Homogenize thoroughly (if the dried material is hard, crush it first before adding 1 mL of Reagent II and homogenizing, or use a homogenizer). Centrifuge at 8,000 g at 4℃ for 10 min, and retain the supernatant for analysis.

Note: Acetone has a strong odor and is recommended to be used in a fume hood.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 530 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Preheat Reagent III and Standard at 37℃ for more than 10 min.
3. Operation table (The following operations are operated in the 1.5 mL EP Tube):

Reagent	Test Tube (μL)	Control Tube (μL)	Blank Tube (μL)	Standard Tube (μL)
Sample	50	50	0	0
Standard	0	0	0	50
Deionized Water	0	50	50	0
Reagent III	50	0	50	50
Mix well				
Concentrated Sulfuric Acid	400	400	400	400
Mix well and incubate in a 95℃ water bath for 5 min, and then cool. Transfer 200 μL to a microglass cuvette or a 96-well plate, and measure the absorbance at 530 nm, recording them as A_{Test}, $A_{Control}$, A_{Blank} and $A_{Standard}$, respectively. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.				

Note: (1) Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. The Standard Tube and Blank Tube need to be prepared only 1-2 times, while each Test Tube requires a corresponding Control Tube. If A_{Test} is less than 0.1, the sample volume can be appropriately increased. If A_{Test} is greater than 2, the sample to be tested needs to be diluted with deionized water (can be diluted 10 or 20 times). (2) Concentrated sulfuric acid is highly corrosive, so please take proper precautions and handle with care.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

Calculation of ISP content:

ISP (mg/g dry weight)=$(C_{Standard}\times V_1)\times \Delta A_{Test}\div \Delta A_{Standard}\div (W\times V_1\div V_2)\times F=$**$0.05\times \Delta A_{Test}\div \Delta A_{Standard}\div W\times F$**

Where: $C_{Standard}$: Concentration of Standard; 0.05 mg/mL; V_1: sample volume added to the reaction system, 0.05 mL; V_2: Reagent II volume added, 1 mL; W: sample dry weight, g; F: sample dilution factor.

Precautions

1. The minimum detection limit is 50 μg/g dry weight.

Typical Data

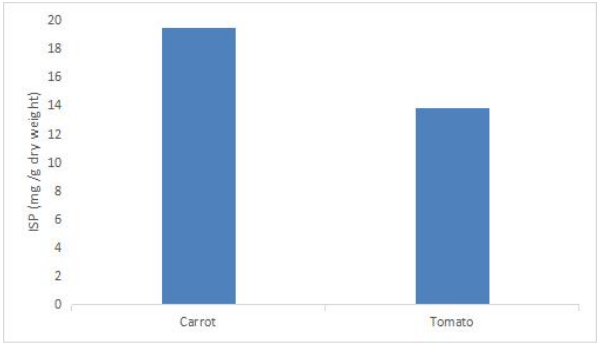

Figure 1. Determination ISP content in Carrot and Tomato by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1583	CheKine™ Mirco Soluble Pectin (WSP) Content Assay Kit
KTB3015	CheKine™ Micro Covalently-Soluble Pectin (CSP) Content Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.