CheKine™ Micro Total Pectin Content Assay Kit

Cat #: KTB1584 Size: 48 T/24 S 96 T/48 S

	Micro Total Pectin Content Assay Kit		
REF	**Cat #:** KTB1584	LOT	**Lot #:** Refer to product label
	Applicable samples: Plant Tissue		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Pectin is an acidic heteropolysaccharide found in plant cell walls, its main chain consists of D-galacturonic acid units linked by α-1,4-glycosidic bonds, with carboxyl groups that can undergo methylesterification or acetylation (or amidation in industrial applications); its side chains contain neutral sugars such as rhamnose, arabinose, galactose, and xylose. Pectin substances are classified into pectinogen (insoluble), water-soluble pectin, and pectic acid; they possess gel-forming, thickening, and emulsifying properties, and are widely utilized in the food, textile, printing and dyeing, tobacco, and metallurgical industries.

The detection principle of this kit is as follows: pectin is hydrolyzed by a dilute acid to yield soluble pectin; this soluble pectin then further reacts with galacturonic acid to form a compound. In a strong acid environment, the galacturonic acid condenses with carbazole to produce a purplish-red compound, which exhibits characteristic absorption at 530 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer Ⅱ	60 mL	120 mL	4℃
Reagent Ⅲ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	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
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge, thermostat water bath
- Deionized water, concentrated sulfuric acid; ethanol absolute
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer Ⅰ: 95% ethanol. That is, 95 mL ethanol absolute and 5 mL deionized water are mixed. **(Required but not provided)**

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

Reagent Ⅰ: Concentrated sulfuric acid. **(Required but not provided)**

Note: Reagent Ⅰ highly corrosive, please handle with extreme caution.

Reagent Ⅱ: Ethanol absolute. **(Required but not provided)**

Working Reagent Ⅲ: Prepared before use. Add 2.1 mL ethanol absolute for 48 T and 4.2 mL ethanol absolut for 96 T to fully dissolve. The prepared reagent can be stored at 4℃, protected from light for 1 month.

Note: Reagent Ⅲ is toxic, so it is recommended to conduct experiments in a fume hood.

Standard: Prepared before use; Add 1 mL deionized water to fully dissolve, that is 5 mg/mL Standard; store at 4℃, protected from light for 1 month.

0.5 mg/mL Standard: Prepare 0.5 mg/mL Standard by diluting 10 μL 5 mg/mL Standard into 90 μL deionized water. 0.5 mg/mL Standard was used for detection.

Notes: Always prepare fresh 0.5 mg/mL Standard per use; Diluted solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When it is time to conduct this experiment, remove the sample from -80℃ and thaw it on ice. However, please note that this process may affect the stability of the sample, and the experimental results may be lower than expected. If it is necessary to simultaneously determine Total Pectin and Protopectin, it is recommended to crush and thoroughly mix the sample before weighing it, in order to minimize the impact of variations in sampling sites on the results.

Plant tissue: Mash the tissue sample, weigh about 0.05 g of the sample, add 1 mL of Extraction Buffer Ⅰ, soak in a constant temperature water bath at 90℃ for 30 min, remove the sample, allow it to cool, then centrifuge at 5,000 g for 10 min at room temperature (25℃), and discard the supernatant. Add 1 mL of Extraction Buffer Ⅰ in the precipitation, repeat the operation, and discard the supernatant after centrifugation. Add 1 mL of Extraction Buffer Ⅱ to the precipitate, put it in a constant temperature water bath at 90℃ for 1 h, remove the mixture, allow it to cool, then centrifuge at 8,000 g for 15 min at room temperature (25℃); collect the supernatant for testing.

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. Sample measurement. (The following operations are operated in the 1.5 mL EP tube)

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)	Control Tube (μL)
Sample	0	0	30	30
Standard（0.5 mg/mL）	0	30	0	0
Reagent Ⅰ	180	180	180	180
Mix well, water bath at 90℃ for 10 min, **and take it out and cool it naturally before opening the cover to prevent the liquid from splashing and burning.**				
Reagent Ⅱ	0	0	0	30
Working Reagent Ⅲ	30	30	30	0
Mix well and let stand at 25℃ for 30 min.				

Deionized water	90	60	60	60

3. Mix well, take 200 μL into a 96-well plate or microglass cuvette. Detect the absorbance at 530 nm. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Control Well is marked as $A_{Control}$; the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Blank Tube and the Standard Tube only need to be done 1-2 times, for each Test Tube, a Control Tube must be prepared. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.01, the sample volume can be appropriately increased. If A_{Test} is greater than 0.8, the sample can be appropriately diluted with Extraction Buffer Ⅱ, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

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 the Total Pectin

Calculated by fresh weight of samples

Total Pectin (mg/g) $=(C_{Standard}\times V_{Standard})\times\Delta A_{Test}\div\Delta A_{Standard}\div(W\times V_{Sample}\div V_{Total\ sample})\times n$**$=0.5\times\Delta A_{Test}\div\Delta A_{Standard}\div W\times n$**

$C_{Standard}$: Standard concentration, 0.5 mg/mL; $V_{Standard}$: standard volume adde, 0.03 mL; V_{Sample}: sample volume added, 0.03 mL; $V_{Total\ sample}$: Extraction Buffer Ⅱ volume added, 1 mL; W: sample weight, g; n: the sample dilution factor.

Typical Data

Examples:

1. Test 0.0501 g orange peel tissue, prepared the sample following the above protocol, dilute the sample 3-fold, and measured with the 96-well microplate:

$\Delta A_{Test}=A_{Test}-A_{Control}=0.9217-0.1383=0.7833$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}=0.4600-0.1123=0.3477$

Calculated by fresh weight of samples, the calculated Total Pectin (mg/g) is $0.5\times\Delta A_{Test}\div\Delta A_{Standard}\div W\times n=67.449$ mg/g.

Recommended Products

Catalog No.	Product Name
KTB1410	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1420	CheKine™ Micro Aspartate Aminotransferase (AST/GOT) Activity Assay Kit
KTB1430	CheKine™ Micro Proline (PRO) Content Assay Kit
KTB1580	CheKine™ Micro Protopectin 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.