CheKine™ Micro Protopectin Content Assay Kit

Cat #: KTB1580 Size: 48 T/96 T

	Micro Protopectin Content Assay Kit		
REF	**Cat #:** KTB1580	LOT	**Lot #:** Refer to product label
	Applicable samples: Plant Tissue		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Pectin is one of the main components of plant cell wall, which is divided into water-soluble pectin and insoluble pectin, namely protopectin. It is widely used in food, textile, printing and dyeing, tobacco, metallurgy and other fields because of its good emulsification, thickening and gelation. CheKine™ Micro Protopectin Content Assay Kit can be used to detect biological samples such as plant tissue. In the kit, protopectin was hydrolyzed into soluble pectin in dilute acid, and further converted into galacturonic acid. The product was condensed with carbazole in strong acid to form a purplish red compound with characteristic absorption at 530 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer II	50 mL	100 mL	4℃
Reagent III	1	1	4℃, protected from light
Standard	1	1	4℃, protected from light

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
- 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 is highly corrosive, please use caution.

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

Reagent III: Prepared before use. Add 1.5 mL deionized water for 48 T and 3 mL eionized water for 96 T to fully dissolve. The prepared reagent can be stored at 4℃, protected from light for 1 month.

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

0.5 mg/mL galacturonic acid Standard preparation: Prepare 0.5 mg/mL galacturonic acid Standard by diluting 10 μL 5 mg/mL trehalose Standard into 90 μL Deionized Water. 0.5 mg/mL galacturonic acid standard was used for detection.

Notes: Always prepare fresh Standards per use; Diluted Std. 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 measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

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, take out 5,000 g after cooling, centrifuge at 25℃ for 10 min, discard the supernatant, add 1 ml of Extraction Buffer I in the precipitation, repeat the operation, and remove the supernatant after centrifugation. Add 1 mL deionized water to the precipitate, take a water bath at 50℃ for 30 min, 5,000 g, centrifuge at 25℃ for 10 min after cooling, and discard the supernatant. Add 1 mL of Extraction Buffer II to the precipitate, put it in a constant temperature water bath at 90℃ for 1 h, take out 8,000 g after cooling, centrifuge at 25℃ for 15 min, and take the supernatant to be measured.

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 Well (μL)	Standard Well (μL)	Control Well (μL)	Test Well (μL)
Sample	0	0	30	30
Standard	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	30	0
Reagent III	30	30	0	30
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 Well and the Standard Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If A_{Test} is greater than 1.0 or ΔA_{Test} is greater than 0.4, 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 protopectin content

Calculated by fresh weight of samples

Protopectin (mg/g fresh weight)=($C_{Standard}$×$V_{Standard}$)×ΔA_{Test}÷$\Delta A_{Standard}$÷(W×V_{Sample}÷$V_{Total\ sample}$)**=0.5×ΔA_{Test}÷$\Delta A_{Standard}$÷W**

$C_{Standard}$: Standard concentration, 0.5 mg/mL; $V_{Standard}$: Added standard volume, 0.02 mL; V_{Sample}: Added the sample volume, 0.02 mL; $V_{Total\ sample}$: Added Extraction Buffer II volume, 1 mL; W: Sample weight, g.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

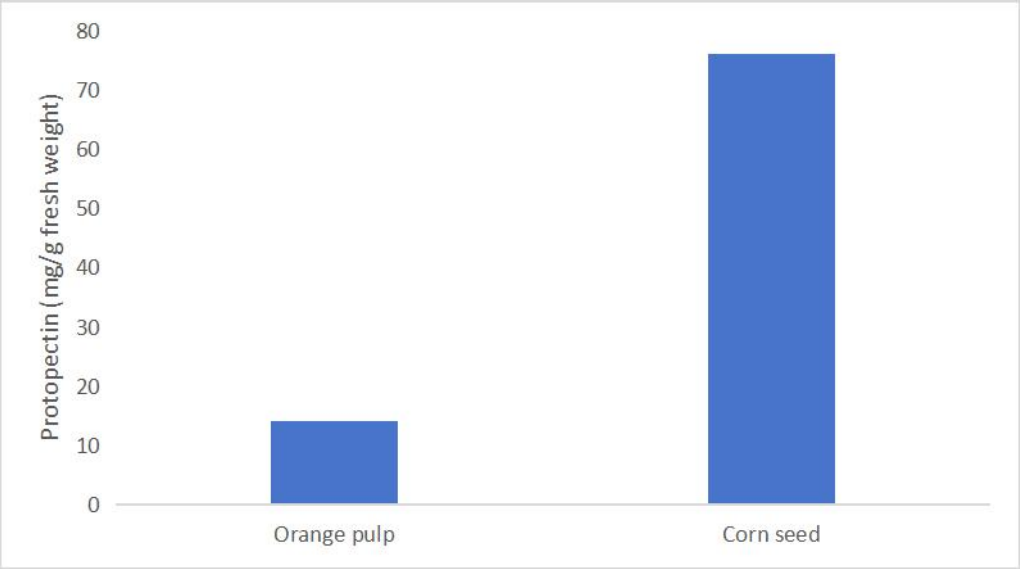

Figure 1. Determination of protopectin content in orange pulp and corn seed by this kit.

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

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.