

Total pectin content Assay Kit

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

Detection instrument: Spectrophotometer/ Microplate reader

Cat No: BC1405

Size: 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Storage
Extract Solution I	Solution 110 mL×2	2-8°C
Extract Solution II	Solution 120 mL×1	2-8°C
Reagent I	Self-provided reagent	-
Reagent II	Solution 3 mL×1	2-8°C
Reagent III	Solution 5 mL×1	2-8°C
Standard	Powder×1	2-8°C

Solution preparation:

Reagent I: Self-provided sulfuric acid, approximately 25 mL, and store at room temperature; A 30mL brown empty bottle is provided in the reagent kit for packaging purposes only. Please label the reagent name on your own.

Standard: 10 mg of galacturonic acid. 0.943 mL of Extract solution II is added to prepare a standard solution of 50 $\mu\text{mol/mL}$ before use.

Product Description:

Pectin is one of the main components of plant cell walls, and is divided into water-soluble pectin and insoluble pectin (original pectin or alkali-soluble pectin). Pectin is a natural polymer compound with good gelling and emulsifying stability. It has been widely used in food, medicine, daily chemical and textile industries.

The original pectin is hydrolyzed into soluble pectin in dilute acid. It and the original soluble pectin is further converted into galacturonic acid. The product is condensed with carbazole in a strong acid to form a purple-red compound, which has a characteristic absorption peak at 530 nm.

Technical index:

Minimum detection limit: 0.096 $\mu\text{mol/mL}$

linear range: 0.25-4 $\mu\text{mol/mL}$

Reagents and Equipment Required but Not Provided.

Spectrophotometer/microplate reader, table centrifuge, water bath, concentrated sulfuric acid (>95%, AR), mortar/homogenizer, micro glass cuvette/96 well flat plate, adjustable pipette and distilled water.

Procedure:

I. Extract Total pectin:

Crush the tissue sample and prepare it in a 1:20 ratio of sample weight (g) to Extraction solution I volume (mL) (it is recommended to take about 0.05g of sample and add 1mL of Extraction solution I). Place it in a 90°C constant temperature water bath for extraction for 30 minutes (wrap the sealing film to prevent bursting). After cooling, centrifuge at 5000g and 25°C for 10 minutes to remove the supernatant. Add 1mL of Extraction solution I to the precipitate and repeat the process once. After centrifugation, remove the supernatant. Add 1mL of Extraction solution II to the precipitate and hydrolyze it in a 90°C constant temperature water bath for 1 hour (wrap the sealing film to prevent bursting). Remove and cool it, centrifuge at 8000g and 25 °C for 15 minutes, and take the supernatant for testing.

II. Determination procedure:

- 1 Preheat the spectrophotometer/microplate reader 30 min, adjust wavelength to 530 nm, set zero with distilled water.
- 2 Preparation of standard solution: Dilute 50 μmol/mL standard solution with Extract solution II to 3、 2.5、 2、 1.5、 1、 0.5 μmol/mL standard solution for use (be careful not to directly detect the absorbance value in this step).
- 3 Operation table:

Reagent name (μL)	Blank tube (B)	Standard tube (S)	Control tube (C)	Test tube (T)
Sample	-	-	25	25
Standard solution	-	25	-	-
Distilled water	25	-	-	-
Reagent I	200	200	200	200
Mix well, leave it at 90 °C for 10min (Wrap the sealing film to prevent bursting), and remove it and cool.				
Reagent II	-	-	25	-
Reagent III	25	25	-	25
Mix well, after standing at 25°C for 30 minutes, pipette 200 μL into a micro glass cuvette or 96-well plate, and measure the absorbance at 530 nm of each tube and record them as A_B , A_S , A_C , and A_T . $\Delta A_S = A_S - A_B$, $\Delta A_T = A_T - A_C$.				

III. Calculation of Total pectin content:

- 1 Drawing of standard curve:
Taking the concentration of each standard solution as the x-axis and its corresponding ΔA_S as the y-axis, draw a standard curve to get the standard equation $y = kx + b$, and bring ΔA into the equation to get x (μmol/mL).
- 2 Calculation of Total pectin content:

Total pectin content ($\mu\text{mol/g weight}$) = $x \times V_{\text{EII}} \div W = x \div W$

V_{EII} : Add the volume of Extract Solution II, 1 mL;

W: Sample weight, g.

Note:

1. Concentrated sulfuric acid is highly corrosive. Pay special attention when operating. After heating and cooling at 90°C , open the lid to prevent liquid splashing and burns.
2. If the absorbance value exceeds the linear range, the sample size can be increased or diluted before proceeding with the measurement.

Examples:

1. Take 0.05g of apple pulp for sample processing, take supernatant, follow the determination procedure to operate, with 96-well plate to calculate: $\Delta A = A_{\text{T}} - A_{\text{B}} = 0.25 - 0.074 = 0.176$, standard curve: $y = 0.5149x - 0.1393$, calculate $x = 0.612$, according with mass of sample to calculate: Total pectin content ($\mu\text{mol/g weight}$) = $x \times 5(\text{dilution ratio}) \div W = 61.2 \mu\text{mol/g weight}$.

Recent Product citations:

- [1] Liang C, Wei C, Wang L, Guan Z, Shi T, Huang J, Li B, Lu Y, Liu H, Wang Y. Characterization of a Novel Creeping Tartary Buckwheat (*Fagopyrum tataricum*) Mutant lazy1. *Front Plant Sci.* 2022 Apr 27;13:815131. doi: 10.3389/fpls.2022.815131. PMID: 35574111; PMCID: PMC9094088.
- [2] Cai Y, Tang C, Lv S, Chen Q, Zhu X, Li X, Qi K, Xie Z, Zhang S, Wang P, Wu J. Elucidation of the GAUT gene family in eight Rosaceae species and function analysis of PbrGAUT22 in pear pollen tube growth. *Planta.* 2023 Feb 28;257(4):68. doi: 10.1007/s00425-023-04103-5. PMID: 36853424.
- [3] Ge L, Lai H, Huang Y, Wang Y, Li Y, Zhu S, Shi Q, Li H, Zhu Y, Zhao N. Comparative evaluation of package types in alleviating textural softening and package-swelling of Paocai during storage: Insight into microbial invasion, cell wall pectinolysis and alteration in sugar and organic acid profiles. *Food Chem.* 2021 Dec 15;365:130489. doi: 10.1016/j.foodchem.2021.130489. Epub 2021 Jun 28. PMID: 34243120.
- [4] Feng J, Li Z, Luo W, Liang G, Xu Y, Chong K. COG2 negatively regulates chilling tolerance through cell wall components altered in rice. *Theor Appl Genet.* 2023 Jan;136(1):19. doi: 10.1007/s00122-023-04261-w. Epub 2023 Jan 21. Erratum in: *Theor Appl Genet.* 2023 Mar 23;136(4):84. PMID: 36680595.
- [5] Wang F, Lu T, Zhu L, Cao A, Xie S, Chen X, Shen H, Xie Q, Li R, Zhu J, Jin X, Li H. Multicopper oxidases GbAO and GbSKS are involved in the *Verticillium dahliae* resistance in *Gossypium barbadense*. *J Plant Physiol.* 2023 Jan;280:153887. doi: 10.1016/j.jplph.2022.153887. Epub 2022 Dec 15. PMID: 36543064.

Related Products:

BC2630/BC2635	Pectinase Activity Assay Kit
BC3680/BC3685	Protopectin Content Assay Kit
BC2660/BC2665	Ploygalacturonase (PG) Activity Assay Kit