# Total pectin content Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Detection instrument:**Spectrophotometer/ Microplate reader

**Cat No:** BC1405

**Size:** 100T/48S

**Components:**

Extract solution 1: 110mL×2, stored at 4°C.

Extract solution 2: 120 mL×1, stored at 4°C.

Reagent 1: 25 mL of concentrated sulfuric acid, provide for oneself.

Reagent 2: 3 mL×1, stored at 4℃.

Reagent 3: 5 mL×1, stored at 4°C and protected from light.

Standard: powder×1, 10 mg of galacturonic acid, stored at 4°C. 0.943 mL of Extract solution 2 is added to prepare a standard solution of 50 μmol/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.

**Required material**

Table centrifuge, spectrophotometer/microplate reader, water bath, concentrated sulfuric acid, mortar/homogenizer, micro glass cuvette/96 well flat bottom plate, adjustable pipette and distilled water.

**Procedure:**

**I. Extract Total pectin:**

The tissue sample is mashed. The ratio of the sample mass (g) and the volume of the Extract solution 1 (mL) is 1: 20 (recommended to take about 0.05 g of sample and add 1 mL of extract solution 1), place it in a 90°C thermostatic water bath, extract for 30 min, remove and cool, centrifuge at 5000g and 25°C for 10 min, remove the supernatant, add 1mL of Extract solution 1 to the precipitate, repeat the operation once, remove the supernatant after centrifugation, add 1 mL of Extract solution 2 to the precipitate, and place at 90°C thermostatic water bath. It is hydrolyzed in a water bath for 1 h. After cooling, it is centrifuged at 8000g and 25°C for 15 min. The supernatant is take for test.

**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 2 to 2、1、0.5、0.25、0.125、0.0625 μ mol/mL standard solution for future use.

3 Add reagents with the following list:

| 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 1 | 200 | 200 | 200 | 200 |
| Mix well, leave it at 90 ℃ for 10min, and remove it and cool. | | | | |
| Reagent 2 | - | - | 25 | - |
| Reagent 3 | 25 | 25 | - | 25 |
| Mix well, after standing at 25°C for 30 minutes, pipette 200 μL into a micro glass cuvette or 96-well flat bottom 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 $\Delta A_S$ 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 (μmol/g fresh weight) = $x \times V_{E2} \div W = x \div W$

$V_{E2}$: Add the volume of Extract Solution 2, 1 mL;

W: Fresh 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 is greater than 1, the sample Extract solution 2 can be appropriately diluted and then measured, and multiplied by the dilution factor in the calculation formula.

**Examples:**

1. Add 0.1g apple pulp to 1mL extract solution and grind thoroughly on ice, take supernatant, follow the determination procedure to operate, with 96-well flat-bottom plates to calculate: ΔA =A(T)-A(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 (μmol/g mass)= x×5(dilution ratio)÷W=61.2 μmol/g mass.

**Related Products:**

BC2630/BC2635 Pectinase Activity Assay Kit

BC3680/BC3685 Protopectin Content Assay Kit

BC2660/BC2665 Ploygalacturonase (PG) Activity Assay Kit