Protopectin Content Assay Kit

Note: Take two or three different samples for prediction before test.
Operation Equipment: Spectrophotometer/microplate reader
Catalog Number: NA0518
Size:100T/48S

Components:
Extract solution I: 80% ethanol, provide for oneself. Take 80 mL of ethanol and add 20 mL of distilled water.
Extract solution II: 50 mL×1, stored at 4℃.
Extract solution III: 70 mL×1, stored at 4℃.
Reagent I: 30 mL of concentrated sulfuric acid (H_2SO_4), **self-prepared reagent**.
Reagent II: 3 mL×1, stored at 4℃.
Reagent III: 5 mL×1, stored at 4℃.
Standard: powder×1, 10 mg of galacturonic acid, stored at 4℃. Before use, add 0.943 mL extract solution III to prepare a standard solution of 50 μmol/mL.

Product Description
Pectin is one of the main components of plant cell wall, which is divided into water-soluble pectin and insoluble pectin. Insoluble pectin is protopectin. Raw pectin is insoluble in water, but it can be decomposed into water-soluble pectin by adding water under the action of acid, alkali, salt and other chemical reagents and enzymes. It is widely used in food, textile, printing and dyeing, tobacco, metallurgy and other fields.
The protopectin is hydrolyzed to soluble pectin under alkaline condition, and further converted to galacturonic acid. The product condensed with carbazole in strong acid to form purplish red compound, with characteristic absorption peak at 530 nm.

Reagents and Equipment Required but Not Provided.
Spectrophotometer/microplate reader, desktop centrifuge, water bath, micro glass cuvette/96 well flat-bottom plate, adjustable pipette, mortar/homogenizer, acetone, concentrated sulfuric acid (H_2SO_4), anhydrous ethanol and distilled water.

Procedure
1. Extraction of protopectin
Take about 0.1 g of sample, add 1 mL of extract solution I, rapidly homogenization at room temperature, water bath at 95℃ for 20 min, cool to room temperature, centrifugate at 4000 ×g for 10 minutes at 25℃, discard the supernatant. Add 1.5 mL of extract solution I and acetone to the precipitate and wash them twice alternately (vortex oscillation for 2 minutes, centrifugate at 4000 ×g for 10 minutes at 25℃, discard

supernatant). The precipitate is the rough cell wall. Add 1 mL of extract solution II (starch removal) to soak for 15 hours, centrifugate at 4000 ×g for 10 minutes at 25℃, discard the supernatant, add 1 mL of extract solution III, and fully homogenize. Centrifugate at 8000 ×g for 10 minutes at 25℃ and take the supernatant for test.

2. Measurement steps

a. Preheat the spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 530 nm and adjust zero with distilled water.

b. Dilute 50 μmol/mL standard solution to 0.8, 0.7, 0.6, 0.5, 0.4, 0.2, 0.1 and 0.05 μmol/mL standard solution for standby.

c. Operation table:

Reagent name (μL)	Blank tube(B)	Standard tube (S)	Contrast tube (C)	Test tube (T)
Sample	-	-	25	25
Standard	-	25	-	-
Distilled water	25	-	-	-
Reagent I	200	200	200	200
Mix well, place at 90 ℃ for 10 minutes, take out and cool down.				
Reagent II	-	-	25	-
Reagent III	25	25	-	25
Mix well, let it stand at 25℃ for 30 minutes, and then take 200 μL in the micro glass cuvette/96 well plate to measure the absorbance value at 530 nm, and record it as A_B, A_S, A_C and A_T respectively. $\Delta A_S=A_S-A_B$, $\Delta A_T=A_T-A_C$.				

III. Calculation of Betaine Content:

1. Drawing of standard curve:

Take ΔA_S as y-axis, standard solution concentration as x-axis, draw standard curve, get standard equation y = kx+b, bring ΔA_T into the equation, get x (mg/mL).

2. Calculation of protopectin content:

protopectin content (μmol/g Fresh weight) $=x\times V_{EIII}\div W=x\div W$.

V_{EIII}: volume of extract solution solution III, 1 mL;

W: Fresh weight of sample, g.

Note:

1. Concentrated H_2SO_4 is highly corrosive, so special attention shall be paid during operation. After heating at 90℃, take it out, cool it and then open the cover to prevent liquid splashing and burning.
2. If ΔA is more than 0.3, the sample can be appropriately diluted with extract solution III and then determined, and multiplied by the dilution multiple in the calculation formula.

Experimental examples:

1. Take about 0.1 g of hibiscus leaves, add 1 mL of extract solution I, rapidly homogenization at room temperature, water bath at 95℃ for 20 minutes, cool to room temperature. Centrifugate at 4000 ×g for

10 minutes at 25℃, discard the supernatant. Add 1.5 mL of extract solution I and acetone to the precipitate and wash them twice alternately (vortex oscillation for 2 minutes, centrifugate at 4000 ×g for 10 minutes at 25℃, discard supernatant). The precipitate is the rough cell wall. Add 1 mL of extract solution II (starch removal) to soak for 15 houes, centrifugate at 4000 ×g for 10 minutes at 25℃, discard the supernatant, add 1 mL of extract solution III, and fully homogenize. Centrifugate at 8000 ×g for 10 minutes at 25℃ and take the supernatant for test. The content is calculated according to the sample mass.

protopectin content (μmol/g Fresh weight)= x÷W=2.628 μmol/g.

Related products:

NA0680/NA0438 Pectinase Activity Assay Kit

NA0329/NA0328 Soluble Pectin Content Assay Kit

NA0679/NA0437 Pectin Lyase Activity Assay Kit