Polysaccharide Content Assay Kit

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

Operation equipment: Spectrophotometer

Cat No: BC6160

Size: 50T/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	Preservation Condition
Reagent I	Liquid 10 mL×1	2-8℃
Standard	Powder×1	2-8℃

Solution Preparation:

Standard solution: Dissolve with 1 mL of distilled water into 10 mg/mL glucose before using, and unused liquid can be stored at 2-8℃ for 4 weeks.

Description:

Polysaccharides are widely found in organisms. They are high-molecular polymers formed by the connection of aldehyde groups and ketone groups through glycosidic bonds. They are one of the four basic substances that constitute life. They have a wide range of functions and have very important and special physiological activities.**The kit is used to determine soluble polysaccharides, excluding starch and cellulose.**

Under the action of concentrated sulfuric acid, the polysaccharide was hydrolyzed into monosaccharides and rapidly dehydrated to form glucaldehyde derivatives, which reacted with phenol to form an orange yellow solution. There was a characteristic absorption peak at 490 nm, because the absorbance value was proportional to the glucose content. Therefore, the polysaccharide content of the sample can be determined by measuring the absorbance value at 490 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, water-bath, tabletop centrifuge, vortex mixer, adjustable pipette, 1 mL glass cuvette, mortar/ homogenizer, ethanol (>99%, AR),concentrated sulfuric acid (>95%, AR) , ice and distilled water.

Operation procedure:

I. Sample preparation

Collecting about 0.1g of tissue, add 1mL of distilled water, grinding on ice bath with homogenizer or mortar, centrifuge at 8000g for 10min at 4℃, take 0.4mL of supernatant, add 1mL of ethanol (supernatant/ ethanol=1:4, V/V), Vortex oscillation mixing for 1min, centrifuge at 12000g for 10min at 4℃, The

supernatant was discarded and 0.4 mL of distilled water was added to fully dissolve the precipitate (the amount of distilled water added was consistent with the amount of the initial supernatant). centrifuge at 8000g for 10min at 4℃, take supernatant and place on ice for test.

Note: If the extraction ratio is changed, it should be noted that: ①The ratio of sample supernatant to anhydrous ethanol is maintained at 1:4 (V/V); ②The amount of the precipitate dissolved by the second addition of distilled water should be consistent with the amount of the supernatant taken by the initial homogenization centrifugation.

II. Determination procedure

1. Preheat spectrophotometer for more than 30 minutes, adjust wavelength to 490 nm and set zero with distilled water.
2. Standard preparation: Dilute the standard to 0.12, 0.08, 0.04, 0.4, 0.02, 0.01 mg/mL with distilled water.
3. Operation table (Add the following reagents to 1.5mL EP tube)

Reagent name (μL)	Test tube (T)	Blank tube (B)	Standard tube(S)
Sample	150	-	-
Distilled water	-	150	-
Standard	-	-	150
Reagent I	150	150	150
concentrated sulfuric acid	750	750	750

Mix well and react in boiling water bath for 20 min (Wrap the sealing film to prevent the lid from opening).After taking out, immediately cool to room temperature, mix well.Take 1 mL to the glass cuvette, and read the absorbance of the test tube, blank tube and standard tube at 490 nm wavelength. Calculate $\Delta A_T=A_T-A_B$, $\Delta A_S=A_S-A_B$. The blank tube and standard curve only need to be measured 1-2 times.

Note : 1. When adding concentrated sulfuric acid, in order to prevent instantaneous burst boiling and cause danger, please add concentrated sulfuric acid slowly along the EP tube wall.

2. It is recommended to use a spiral port or an EP tube with a latch to prevent a burst cover, or a syringe needle can be used to place a small hole in the ordinary EP tube cover (still need to wrap the sealing film).

III. Calculate of Polysaccharide Content

1. Standard curve

The standard curve is established according to the concentration of the standard tube (x, mg/mL) and the absorbance ΔA_S (y, ΔA_S). According to the standard curve, the ΔA_T (y) is brought into the formula to calculate the sample concentration (x, mg/mL).

2. Calculate by sample mass

Polysaccharide Content (mg/g mass)$=x\times V_T\div(W\div V_E\times V_T)\times F=x\div W\times F$

V_T: Sample volume , 0.15mL;

V_E: Volume of distilled water added , 1mL;

W: Sample mass, g;

F: Sample dilution multiple.

Note:

1. Before the experiment, 1-2 samples were used for pre-experiment to ensure that 0.04< ΔA< 1; if A_T >1, it is recommended that the last step of the sample supernatant is diluted with distilled water and then determined. Note the synchronous modification calculation formula.

1. Experimental example:

1. Take 0.1040g of *Hosta plantaginea (Lam.) Aschers*. leaves, add 1 mL of distilled water, grind the homogenate with ice bath, 0.4 mL of the supernatant was removed by centrifugation, and add 1 mL of ethanol, mix well thoroughly for 1min. After centrifugation, discard the supernatant and leave the precipitation add 0.4 mL of distilled water. Mix thoroughly and then centrifuge. The supernatant was diluted 10 times and followed the measurement steps. The ΔA= A_T-A_B=1.045-0.231=0.814 measured by 1 mL glass cuvette, standard curve: y=8.0046x+0.0147, R^2=0.9975, calculate x=0.0995, and polysaccharide content is calculated according to the sample mass:

   polysaccharide content (mg/g mass) =x÷W×F=9.88 mg/g mass.

2. Take 0.1040g of fresh peach leaves, add 1 mL of distilled water, grind the homogenate with ice bath, 0.4 mL of the supernatant was removed by centrifugation, and add 1 mL of ethanol, mix well thoroughly for 1min. After centrifugation, discard the supernatant and leave the precipitation add 0.4 mL of distilled water. Mix thoroughly and then centrifuge. The supernatant was diluted 10 times and followed the measurement steps. The ΔA=A_T-A_B=0.638-0.231=0.407 measured by 1 mL glass cuvette, standard curve: y=8.0046x+0.0147, R^2=0.9975, calculate x=0.0487, and polysaccharide content is calculated according to the sample mass:

   polysaccharide content (mg/g mass) =x÷W×F=1.85 mg/g mass.

Related Products:

BC2500/BC2505	Glucose Content Assay Kit
BC0030/BC0035	Plant Soluble Sugar Content Assay Kit
BC2710/BC2715	Total Carbohydrate Content Assay Kit
BC0230/BC0235	Reducing Sugar Content Assay Kit