Sucrose HPLC Assay Kit

Note: Choose 2-3 sample with large expected differences for prediction before formal determination.

Detection Equipment: High performance liquid chromatography(HPLC)

Catalog Number: BC2464

Size: 50T/48S

Components:

Reagent I: Liquid 2.5mL ×1, Storage at 4 ℃.

Reagent II: Powder ×1, Storage at 4 ℃. Before use, add 2.5 mL distilled water to dissolve thoroughly.

Standard product: Powder ×1, Storage at 4℃ away from light. Before use, each branch was prepared with 5 mL distilled water to form 20 mg/mL mannitol standard solution and storage at 4℃. Use now and match now.

Description:

Sucrose (table sugar), a disaccharide, is formed by the condensation and dehydration of the semi-acetal hydroxyl group of one molecule of glucose and the semi-acetal hydroxyl group of one molecule of fructose. Sucrose has a sweet taste and is found almost universally in the leaves, flowers, stems, seeds and fruits of the plant kingdom. It is especially abundant in sugar cane, sugar beet and maple SAP.

The sucrose content was determined by differential refraction detector.

Reagents and Equipment Required but Not Provided:

High performance liquid chromatograph (Agilent ZORBAX Carbohydrate chromatography column (4.6 × 250 mm, sugar analysis column), differential refraction detector (RID)), table centrifuge, adjustable pipette, mortar/homogenizer, EP tube, needle filter (organic system, water system, 0.45μm), Syringe, filter, filter membrane (organic system, water system), brown sample bottle (1.5mL), acetonitrile (chromatographic pure), ultra-pure water.

Preparation before The Experiment:

1. The chromatographic pure acetonitrile was filtered by organic filter membrane and ultrapure water was filtered by aqueous filter membrane, and 75% acetonitrile-water solution was prepared by the filtered acetonitrile and ultrapure water as the mobile phase. (The acetonitrile was filtered by 0.45 μm organic filter membrane, and the ultrapure water was filtered by 0.22 μm water filter membrane).
2. The prepared mobile phase was sonicated for 20 min to remove the gas in the solvent.
3. Preparation of standard substance: 20 mg/mL sucrose standard solution was diluted into 10mg/mL, 5 mg/mL, 2.5 mg/mL and 1.25 mg/mL sucrose standard solution with distilled water. (The concentration of the prepared standard is for reference only and can be adjusted according to the actual sample concentration). Seal and stored in the dark at 4℃, filtered into brown injection bottle by water needle type filter before testing (please put it in room temperature before testing).

Protocol:

I. Sample Extraction

1. Low Protein Content Sample (plant tissue, etc.)

Solid sample: The tested sample were fully ground, added into 2 mL EP tubes according to the ratio of mass (g): volume of distilled water (mL) : 1:10~20 (it is recommended to weigh 0.1 g sample and add 1 mL distilled water), sealed, mixed evenly, and then put into an ultrasonic cleaner, ultrasonic extraction for 30 min at room temperature. After centrifugation at 15000 rpm for 15 min, the supernatant was taken (if necessary, the filter residue was extracted again, and the supernatant was combined), filtered into a brown injection bottle through a water needle type filter, and tested.

Liquid sample: According to the ratio of sample volume (mL): distilled water volume (mL)1:10~100 (it is recommended to weigh 50 μL of sample and add 0.95 mL of distilled water), add it to 1 mL EP tube, seal it, mix it well, and centrifuge at 15000 rpm for 10 min. The supernatant was filtered into a brown injection bottle using a water needle filter and tested (if there was still turbidity in the supernatant, the supernatant could be diluted and centrifuged again).

2. Protein-rich sample (animal tissues, etc.) : If the sample contains a lot of protein, after extraction or dilution according to the above methods, take 900 μL supernatant, add 50 μL reagent 1, 50 μL reagent 2, let it stand for 10 min, centrifuge at 15000 rpm for 15 min, take the supernatant, filter it into a brown injection bottle through a water needle filter, and be tested.

3. Cell sample: According to the cell number (10^4): distilled water volume (mL)=1000:1 ratio (it is recommended to weigh 10 million cells and add 1 mL distilled water), add them into 1 mL EP tubes, seal, mix well with full vibration, and then put them into an ultrasonic cleaner, and extract them by ultrasound at room temperature for 30 minutes. After centrifugation at 15000 rpm for 10 min, the supernatant was filtered into a brown injection bottle using a water needle filter and tested (if there was still turbidity in the supernatant, the supernatant could be diluted and centrifuged again).

II. Measurement Steps

1. Turn on the computer, turn on the switch button of each module of the liquid chromatograph, install the chromatographic column, open the software, set the injection volume as 10 μL, column temperature as 35℃, flow rate as 1 mL/min, detection cell temperature as 35℃ in the method group, elution program as shown in the following table, departure time as 20 min, and save the method group after setting.
2. Use the corresponding mobile phase to clean the column, use the prepared mobile phase to balance the column, and start adding sample after the baseline is stable.
3. Test the standard solution to be tested, the injection volume is 10 μL, sucrose can be separated within 20 minutes, and the retention time of sucrose is about 15 minutes (the differences in column, mobile phase, temperature, etc., can cause the deviation of retention time, which is only used as a reference).
4. Test the sample solution to be tested with an injection volume of 10 μL and detect the peak area of sucrose at the corresponding retention time. Step 2 was repeated for the next sample.

III. Calculations

The standard curve of sucrose was drawn with the standard concentration (mg/mL) as the abscissa and the peak area as the ordinate. The peak area of the sample was substituted into the

standard curve to calculate the concentration x(mg/mL) of sucrose in the extract.

1. Almost no protein sample

Solid sample: Sucrose content (mg/g) $=x \times V_{sample\ total} \div W = x \div W$

$V_{sample\ total}$: the volume of distilled water, 1 mL; W: sample quality, g; sample tested after dilution need to be multiplied by the corresponding dilution before calculation.

Liquid sample: Sucrose content (mg/mL) $= x \times V_{total} \div V_{sample} = 20x$

V_{sample}: the total volume of liquid sample added, 0.05 mL; V_{total}: total volume of liquid sample and added water, 1 mL.

2. Protein-rich sample

Solid sample: Sucrose content (mg/g) $= x \times 1.111 \times V_{sample\ total} \div W = 1.111x \div W$

Liquid sample: Sucrose content (mg/mL) $= x \times 1.111 \times V_{total} \div V_{sample} = 22.222x$

1.111: dilution ratio, (900μL+50μL+50μL) ÷ 900μL =1.111; $V_{sample\ total}$: the volume of distilled water, 1 mL; W: sample quality, g; V_{total}: total volume of liquid sample and added water, 1 mL; V_{sample}: volume of liquid sample added, 0.05 mL.

3. Cell sample: Sucrose content (mg/10^4) $=x \times V_{total} \div$ cell number $=x \div$ cell number

$V_{sample\ total}$: the volume of distilled water, 1 mL; Cell number: number of cells added, 10^4.

Note:

1. After the test, close the column temperature box and the detector heating system to cool down, and rinse according to the type of column to prevent damage to the chromatographic column.
2. The dilution of the standard should be determined according to the concentration of sucrose in the sample. The peak area of sucrose in the sample must be within the peak area of the standard solution of different concentrations, and the dilution of the standard is only a reference. If the sucrose concentration in the sample is too high, it is recommended to dilute it before testing.
3. If the sample size is too large, it is recommended to test the standard solution once a day (one standard solution is enough) to determine the corresponding retention time. All the solutions to be tested must be placed at room temperature before testing.
4. If necessary, blank sample can be detected once, and the influence of reagent factors can be deducted.
5. The sample tested after dilution need to be multiplied by the corresponding dilution before calculation.