Glucose Content 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 : BC2504

Size: 50T/48S

Components:

Reagent I: Liquid 2.5 mL ×1, Storage at 2-8℃.

Reagent II: Powder ×1 , Storage at 2-8 ℃. Dissolve thoroughly by adding 2.5 mL distilled water before application.

Standard: Powder ×1 , Storage at 2-8 ℃. A standard solution of 20 mg/mL glucose standard solution was prepared by adding 5 mL distilled water before use, and the solution was sealed and storage at 2-8℃.

Description:

Glucose, the molecular formula C6H12O6, is one of the most widely distributed and important monosaccharides in nature, and it is apolyhydroxy aldehyde. Pure glucose is a colorless crystal, sweet but not as sweet as sucrose. Natural glucose solution rotates to the right, so it belongs to "dextrose".

The glucose content was determined by differential refraction detector.

Reagents and Equipment Required but Not Provided:

High performance liquid chromatograph (Agilent ZORBAX Carbohydrate column (4.6×250 mm, sugar analysis column)), differential refractive detector (RID), desktop centrifuge, adjustable pipet gun, mortar/homogenizer, EP tube, needle filter (organic system, water system, 0.45 μm), syringe, suction filter, filter membrane (organic system, water system), brown injection bottle (1.5 mL), acetonitrile (chromatography pure), ultra-pure water, ultrasonic cleaner.

Preparation before The Experiment:

1. Chromatographic pure acetonitrile was filtered by organic filter membrane and ultrapure water was filtered by aqueous filter membrane. 85% 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 glucose standard solution was diluted into 10mg/mL, 5 mg/mL, 2.5 mg/mL and 1.25 mg/mL glucose standard solution with distilled water. (The prepared standard concentration is for reference only and can be adjusted according to the actual sample concentration). Sealed and stored in the dark at 4℃, filtered into brown injection bottle with organic needle type filter before testing (please put it in room temperature before testing).

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Protocol:

I. Sample Extraction

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

1) 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, and ultrasonic extracted for 30 min at room temperature. The sample were centrifuged at 15000 rpm for 15 min at room temperature, and the supernatant (if necessary, the filter residue was extracted again, and the supernatant was combined) was filtered into a brown injection bottle through a water needle type filter, and then tested.

2) 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 into 2 mL EP tube, seal it, mix it well, and centrifuge at 15000 rpm for 10 min at room temperature. 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.)

1) 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, and ultrasonic extracted for 30 min at room temperature. After centrifugation at 15000 rpm for 15 min at room temperature, 900 μL supernatant was taken, 50 μL reagent 1 and 50 μL reagent 2 were added, left for 10 min, centrifuged at 15000 rpm for 15 min at room temperature, and the supernatant was filtered into a brown injection bottle using a water needle filter. To be tested (if there is still turbidity in the supernatant, it can be diluted and centrifuged again).

2) Serum (plasma) and other liquid sample: According to the ratio of sample volume (mL): distilled water volume (mL)1:1 to 10 (it is recommended to weigh 500 μL sample and add 500 μL distilled water) into 2 mL EP tube, seal, mix well, centrifuge at 15000 rpm for 10 min at room temperature, take 900 μL supernatant, add 50 μL reagent one, and then remove the supernatant. 50 μL reagent 2 was left for 10 min, centrifuged at 15000 rpm for 15 min at room temperature, and the supernatant was filtered into a brown injection bottle using a water needle filter to be tested (if there was still turbidity in the supernatant, the supernatant could be diluted and centrifuged again).

3. Cell sample

According to the ratio of cell number (10^4): distilled water volume (mL)=1000:1 (it is recommended to weigh 10 million cells and add 1 mL distilled water), add them into 1 mL EP tubes, seal, mix evenly, 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 of 10 μL in the

method group, the column temperature: 35℃, the flow rate: 1 mL/min, the temperature of the detection cell: 35℃, and the time-out time: 27 min.

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. To detect the standard solution to be tested, the injection volume is 10 μL, the glucose can be separated within 27 min, and the retention time of glucose is about 24 min (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, and the injection volume is 10 μL, and the peak area of glucose is detected at the corresponding retention time. Step 2 was repeated for the next sample.

III. Calculations

The standard curve of glucose 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 glucose in the extract.

1. Almost no protein sample

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

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

Liquid sample: Glucose 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 versus added distilled water, 1 mL.

2. Protein-rich sample

Solid sample: Glucose content (mg/g) $= x \times V \div (W \div V_{extraction} \times V_{supernatant}) = 1.111x \div W$

Liquid sample: Glucose content (mg/mL) $= x \times V \div (V_{sample} \div V\ total \times V_{supernatant}) = 2.222x$

V: the total volume of the second centrifugation supernatant, 900μL+50μL+50μL=1 mL; $V_{extraction}$: total volume of distilled water added, 1 mL; $V_{supernatant}$: The volume of supernatant for protein-rich sample, 0.9 mL; V_{total}: total volume of distilled water and liquid sample added, 1 mL; W: sample quality, g; V_{sample}: total volume of liquid sample added, 0.5 mL.

3. Cell sample

The amount of glucose (mg/10^4 cells) $= x \times V_{total} \div N = x \div N$

V_{total}: Add distilled water volume, 1 mL; N: number of cells added, in units of 10^4.

Notes:

1. After the test is completed, close the column temperature box and the detector heating system to cool down, and then rinse according to the type of column specification to prevent damage to the chromatographic column.
2. The dilution of the standard should be determined according to the concentration of glucose in the sample. The peak area of glucose in the sample must be within the peak area of the standard solution of different concentrations. If the glucose concentration in the sample is too high, it is recommended to dilute the sample before testing.
3. If the sample size is too large, it is recommended to test the standard solution once a day (one

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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.
6. This kit is not suitable for the detection of sample containing mannitol, such as sea algae sample.