Mannitol Content HPLC Assay Kit

Note: **It is necessary to predict 2-3 large difference samples before the formal determination**.

Operation Equipment: High performance liquid chromatography

Catalog Number: BC4604

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	Size	Preservation Condition
Reagent I	Liquid 2.5mL×1	2-8℃
Reagent II	Powder ×1	2-8℃
Standard	Powder ×1	2-8℃

Solution Preparation:

1. Reagent II: Dissolve with 2.5 mL ultra-pure water before use.
2. Standard: Dissolve with 5 mL ultra-pure water to prepare 20mg/mL mannitol standard solution before use.

Product Description:

Mannitol has a sweet taste similar to sucrose. It has the effect of diuresis and detumescence and is helpful for the treatment of renal failure, senile edema, drug poisoning.

Mannitol content is determined by differential refraction detector.

Reagents and Equipment Required but Not Provided:

High performance liquid chromatograph (Agilent ZORBAX NH_2 column (4.6×250 mm, amino column)), differential refractive detector (RID), desktop centrifuge, adjustable pipette, 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.

Preparation before the experiment:

1. The chromatographic pure acetonitrile was filtered by organic filter membrane and ultra-pure water was filtered by aqueous filter membrane, and 75% acetonitrile-aqueous solution was configured by the extracted acetonitrile and ultra-pure water as mobile phase. (Acetonitrile was pumped with 0.45μm organic filter membrane, and ultrapure water was pumped with 0.22μm 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 mannitol standard solution was diluted into 10mg/mL, 5 mg/mL, 2.5 mg/mL and 1.25 mg/mL mannitol standard solution with ultra-pure water. (The prepared standard concentration is for reference only and can be adjusted according to the actual sample

concentration). Seal and store at 2-8℃ in the dark. Filter into brown injection bottle by water needle type filter before testing (please put it in room temperature before testing)

Procedure:

I. Extraction of mannitol:

1. Low Protein Content Samples:

1) Solid samples: The tested sample were fully ground, added into 2 mL EP tubes according to the ratio of mass (g): ultra-pure water volume (mL)1:10~20 (it is recommended to weigh 0.1 g sample and add 1 mL ultra-pure water), sealed, mixed evenly, and then put into an ultrasonic cleaner, ultrasonic extraction at room temperature for 30 minutes. After centrifugation at 15000 rpm for 15 min, the supernatant was taken and 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.

2) Liquid samples: According to the ratio of sample volume (mL): ultra-pure water volume (mL)1:10~100 (it is recommended to weigh 50 μL of sample and add 0.95 mL of ultra-pure water), add it to 1.5 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 samples:

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 I, 50 μL Reagent II, 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.

II. Measurement steps:

1. Turn on the computer, turn on the switch buttons of each module of the liquid chromatograph, install the chromatographic column, and open the software. In the method group, set the sample size to 10μL, column temperature to 35℃, flow rate to 1 mL/min, detection pool temperature to 35℃, sampling time to 25 min, and the preservation method group was set.
2. Use the corresponding mobile phase to clean the column, use the prepared mobile phase balance column, and start adding sample after the baseline is stable.
3. Test the standard solution to be measured, the sample size is 10μL, the mannitol can be separated within 25 min, and the retention time of mannitol is about 19 min (The difference of column, mobile phase, temperature, etc., may cause the deviation of the retention time, for reference only).
4. Test the sample solution to be measured, the injection volume is 10 μL, and the peak area of mannitol is detected at the corresponding retention time.

III. Calculation of Mannitol Content

The standard curve of mannitol 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 mannitol in the extract.

1. Low Protein Content Samples

1) Solid samples

Mannitol content (mg/g) =x ×Ve ÷W×F=2x÷W×F

Ve: volume of ultra-pure water, 2 mL; W: Sample weight, g; F: Dilution factor.

2) Liquid samples

Mannitol content (mg/mL) =x ×Vt ÷Vs ×F=20x×F

Vt: volume of sample and ultra-pure water during extraction, 1 mL; Vs: added volume of sample, 0.05 mL; F: Dilution factor.

2. Protein-rich samples

1) Solid samples

Mannitol content (mg/g) =x ×Vs1÷(Vu ÷Ve1 ×W)×F=2.222x÷W×F

2) Liquid samples

Mannitol content (mg/mL) = x ×Vs1÷(Vu ÷Ve2 ×Vs2)×F =22.222x×F

Vs1: total volume of sample, 1 mL; Vu: volume of supernatant during extraction, 0.9 mL; Ve1: added ultra-pure water volume of solid samples, 2 mL; Ve2: total volume of liquid sample and ultra-pure water, 1 mL; Vs2: added volume of liquid sample, 0.05 mL; W: Sample weight, g; F: Dilution factor.

Note:

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