Xylose Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: High performance liquid chromatography

Catalog Number: BC4394

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 50 mL×1	2-8℃
Reagent II	Liquid 25 mL×1	2-8℃
Standard	Powder×1	2-8℃

Solution Preparation:

1. Filter the chromatographically pure acetonitrile through a 0.45 μm organic solvent filter membrane, and filter the ultrapure water through a 0.22 μm aqueous filter membrane. Then, use the filtered acetonitrile and ultrapure water to prepare a 75% acetonitrile-water solution as the mobile phase.
2. Ultrasonicate the filtered Mobile Phase A for 20 minutes to remove air bubbles.
3. Preparation of Standard: Before use, add 5 mL of distilled water to prepare a 20 mg/mL xylose standard solution, and store it under seal at 4°C.The 20 mg/mL xylose standard solution was diluted with distilled water to 10 mg/mL, 5 mg/mL, 2.5 mg/mL and1.25 mg/mL xylose standard solutions, respectively (The prepared standard concentrations are for reference only and can be adjusted according to the actual sample concentration). Store (sealed) at 4°C in the dark, before testing, filter the solution through an organic solvent syringe filter into a brown sample vial and allow it to reach room temperature before measurement.

Product Description:

Xylose is a component of xylan, and natural D-xylose exists in plants in the form of polysaccharides. Xylose is also found in animal heparin, chondroitin, and glycoproteins, where it serves as the linkage unit between the sugar chain and serine (or threonine) in some glycoproteins.

The xylose content is determined using a differential refractive index detector(RID).

Reagents and Equipment Required but Not Provided:

High-performance liquid chromatography (HPLC) instrument (Agilent ZORBAX Carbohydrate Column (4.6×250 mm, specifically for sugar analysis)), refractive index detector (RID), benchtop centrifuge, adjustable pipette, mortar/homogenizer, EP tubes, syringe filters (organic and aqueous, 0.45

μm), syringes, filtration apparatus, filter membranes (organic and aqueous), brown sample vials (1.5 mL), acetonitrile (chromatographically pure), and ultrapure water.

Operation procedure

I. Extraction of xylose:

Solid samples: Grind the test sample thoroughly and add it to a 2 mL EP tube at a ratio of mass (g) to reagent one volume (mL) of 1:10~20 (it is recommended to weigh 0.1 g of sample and add 1 mL of reagent one). Seal the tube, mix well, and incubate in boiling water for 1 hour. Allow it to cool to room temperature. Centrifuge at 15,000 rpm for 15 minutes, then take 500 μL of the supernatant and add 500 μL of reagent two. Mix well, centrifuge again at 15,000 rpm for 15 minutes, and filter the solution through an aqueous syringe filter into a brown sample vial for testing.

Liquid samples: Add the sample to a 1 mL EP tube at a ratio of sample volume (mL) to distilled water volume (mL) of 1:10~100 (it is recommended to pipette 50 μL of sample and add 0.95 mL of distilled water). Seal the tube, mix thoroughly by shaking, and centrifuge at 15,000 rpm for 10 minutes. Filter the supernatant through an aqueous syringe filter into a brown sample vial for testing (if the supernatant is still turbid, it can be diluted and centrifuged again).

II. Determination procedure:

1. Turn on the computer, switch on all modules of the liquid chromatograph, install the chromatographic column, open the software, set the injection volume to 10 μL in the method group, set the column temperature to 35℃, the flow rate to 1 mL/min, the detection cell temperature is set to 35°C, and the run time to 10 minutes. Save the method group after setting.
2. Clean the column with the appropriate mobile phase, equilibrate the column with the prepared mobile phase, and start adding samples after the baseline has stabilized.
3. Detect the standard solution to be tested with an injection volume of 10 μL. Xylose can be separated within 10 minutes, and the retention time of xylose is around 7 minutes (the retention time may vary depending on factors such as the system, column, pH of the mobile phase, and temperature, and is only provided as a reference).
4. Detect the sample solution to be tested with an injection volume of 10 μL, and measure the peak area of xylose at the corresponding retention time.Repeat step 2 before testing the next sample.

III. Calculation:

Plot a standard curve for xylose with the concentration of the standard solution (mg/mL) as the abscissa and the peak area as the ordinate. Substitute the peak area of the sample into the standard curve to calculate the concentration x (mg/mL) of xylose in the extraction solution.

Solid samples:The content of xylose (mg/g mass) = $x \times V_T \div W = 2x \div W$

V_T: The total volume of the samples, 2 mL;

W: Sample weight(g). For samples tested after dilution, multiply by the corresponding dilution factor before calculation.

Liquid samples:The content of xylose (mg/mL) = $x \times V_T \div V_S = 20x$

V_S: The total volume of the liquid sample added, 2 mL;

V_T: The total volume of the liquid sample and distilled water., 1 mL; For samples tested after dilution, multiply by the corresponding dilution factor before calculation.

Note:

1. After the test is completed, turn off the column oven and detector heating system to allow them to cool down. Rinse the column according to the specifications for its type to prevent damage to the chromatographic column.
2. The dilution factor of the standard solution should be determined based on the concentration of xylose in the sample. The peak area of xylose in the sample must fall within the range of peak areas obtained from standard solutions of different concentrations. The suggested dilution factor for the standard solution is only a reference. If the concentration of xylose in the sample is too high, it is recommended to dilute it before measurement.
3. If the sample volume is too large, it is advisable to test a standard solution (just one) once a day to confirm the corresponding retention time. All solutions to be tested should be brought to room temperature before testing.
4. If necessary, a blank sample can be tested to eliminate the influence of reagents.

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