Technical Manual

D-Xylose Colorimetric Assay Kit

- **Catalogue Code: MAES0031**
- **Size: 100 Assays**
- **Research Use Only**

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

0.007-4 mmol/L

Sensitivity:

0.007 mmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

D-Xylose is a sugar first isolated from wood, and named for it. D-Xylose is classified as a monosaccharide of the aldopentose type, which means that it contains five carbon atoms and includes an aldehyde functional group. It is derived from hemicellulose, one of the main constituents of biomass. Like most sugars, it can adopt several structures depending on conditions. With its free aldehyde group, it is a reducing sugar.

D-xylose can be eliminated by kidney. Its incomplete absorption allows for its possible use as a absorption test. A number of studies have utilized D-xylose absorption as an investigative tool to study small intestinal function and renal function in a variety of clinical settings

3. Intended Use

This kit can be used to measure the D-xylose content in animal serum, plasma and urine samples.

4. Detection Principle

D-xylose can produce furfural by dehydration in strong acid solution. The generated furfural reacts with Phloroglucinol to form pink compounds. The content of D-xylose can be calculated by colorimetric assay at 554 nm.

5. Kit components & storage

Item	Specification	Storage
Phloroglucinol	60 mL × 6 vials	2-8°C, 6 months, avoid direct sunlight
Standard solution (13.3 mmol/L)	1 mL × 1 vial	2-8°C, 6 months
Standard diluent	10 mL × 1 vial	2-8°C, 6 months

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (554 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

1. This experiment must be done in a glass test tube, not in an EP tube or other test tube.

2. The temperature of water bath should be maintained above 95°C, then cooled to room temperature with running water immediately.

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **standard solution (1.33 mol/L):** Dilute the standard solution (13.3 mmol/L) with standard diluent at a ratio of 1:9 and mix fully. It can be store at 2-8°C for 3 months with avoid direct sunlight.

8. Sample Preparation

1. Serum sample:
Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 700-1000 g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Urine:
Collect fresh urine and centrifuge at 10000 g for 15 min at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.007-4 mmol/L).

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 554 nm

10. Operation Steps

1. **Blank tube:** add a* mL of double-distilled water into a 10 mL glass tube.
   Control tube: add a* mL of sample which not treated with D-xylose into a 10 mL glass tube.

2. **Standard tube:** add a* mL of standard solution (1.33 mmol/L) into a 10 mL glass tube.
   Sample tube: add a* mL of sample which treated with D-xylose into a 10 mL glass tube.
   Note: a* refers to the volume of double-distilled water, 1.33 mol/L standard solution or sample.

3. Reference sample volume: Serum is 30 μL, urine is 50 μL.

4. Add 3 mL of phloroglucinol into each tube.

5. Incubate the tubes at 100°C (boiling water bath) for 4 min exactly. Take the tubes out and cool with running water immediately.

6. Set to zero with double-distilled water and measure the OD values of each tube at 554 nm with 1 cm optical path cuvette.

7. **Notes:** Some of the reagents are irritating and should be operated in the ventilation cabinet.

Operation Table

	Blank tube	Control tube	Standard tube	Sample tube
Double-distilled water (mL)	a*			
Sample which not treated with D-xylose (mL)		a*		
1.33 mol/L Standard solution (mL)			a*	
Sample which treated with D-xylose (mL)				
Phloroglucino (mL)	3	3	3	
Mix fully and incubate the tubes at 100**°C** (boiling water bath) for 4 min exactly. Take the tubes out and cool with running water immediately. Set to zero with double-distilled water and measure the OD values of each tube at 554 nm with 1 cm optical path cuvette.				

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11. Calculations

$$\text{D-xylose content } (mmol/L) = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

ΔA_1: OD_{sample}-$OD_{Control}$

ΔA_2: $OD_{standard}$-OD_{blank}

c: The concentration of standard, 1.33 mmol/L

f: Dilution factor of sample before test

12. Performance Characteristics

Detection Range	0.007-4 mmol/L
Sensitivity	0.007 mmol/L
Average recovery rate (%)	103
Average inter-assay CV (%)	4.5
Average intra-assay CV (%)	2.2

Analysis

Take 30 µL of human serum, carry the assay according to the operation table.

The results are as follows:

the average OD value of blank is 0.043, the average OD value of standard is 0.449, the average OD value of control is 0.067, the average OD value of sample is 0.165, and the calculation result is:

D-xylose content (mmol/L) = [(0.165-0.067) ÷ (0.449-0.043)] ×1.13
= 0.32 mmol/L

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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