D-Xylose Colorimetric Assay Kit

Catalog No: E-BC-K018-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 48 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.007 mmol/L

Detection range: 0.007-4 mmol/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲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

▲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.

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Phloroglucinol	60 mL × 6 vials	2-8℃ , 6 months, shading light
Reagent 2	13.3 mmol/L D-Xylose Standard	1 mL × 1 vial	2-8℃ , 6 months
Reagent 3	Standard Diluent	10 mL × 1 vial	2-8℃ , 6 months
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Spectrophotometer, Micropipettor, Vortex mixer, Water bath.

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), Glass test tubes (10 mL)

Reagents

Double distilled water, Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

▲Precautions

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

▲The key points of the assay

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℃ , then cooled to room temperature with running water immediately.

Pre-assay preparation

▲Reagent preparation

Preparation of 1.33 mol/L standard solution:

Dilute the reagent 2 with reagent 3 at a ratio of 1:9 and mix fully. It can be store at 4℃ for 3 months with shading light.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.007-4 mmol/L).

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	554 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating 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.
Standard tube: add a* mL of 1.33 mmol/L standard solution 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.

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

(2) Add 3 mL of Reagent 1 into each tube.

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

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

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)				a*
Reagent 1 (mL)	3	3	3	3
Mix fully and incubate the tubes at 100℃ (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.				

▲Calculation

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

Note:

ΔA_1: $OD_{Sample} - OD_{Control.}$

ΔA_2: $OD_{Standard} - OD_{Blank.}$

c: The concentration of standard, 1.33 mmol/L.

f: The dilution factor of sample before tested.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

Appendix I Performance characteristics			
Detection range	0.007-4 mmol/L	Average intra-assay CV (%)	2.2
Sensitivity	0.007 mmol/L	Average inter-assay CV (%)	4.5
Average recovery rate (%)	103		

▲Example analysis

For human serum, take 0.03 mL human serum sample and carry the assay according to the operation table.

The results are as follows:

he 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

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . 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℃ for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4℃ . 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℃ for a month.

▲ Urine

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

▲ Notes for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.