（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K018-S

Specification：50 Assays (24 samples)/ 100 Assays (48 samples)

Measuring instrument：Spectrophotometer (554 nm)

Detection range：0.007-4 mmol/L

Elabscience® D-Xylose Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

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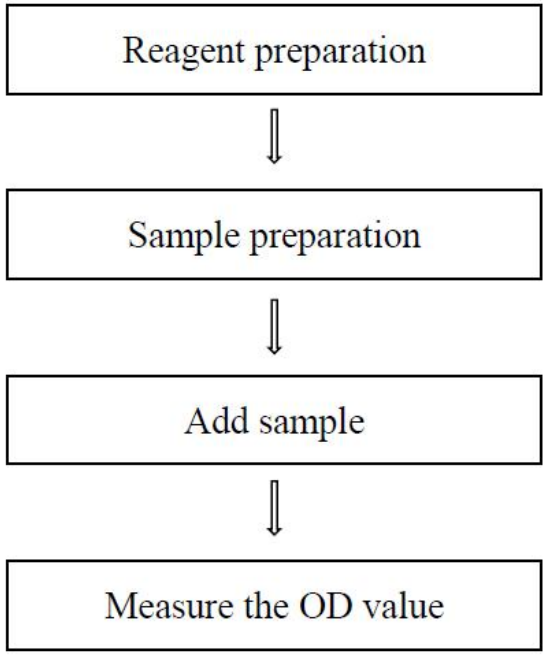
Assay summary



Intended use

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

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	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Phloroglucinol	60 mL × 3 vials	60 mL × 6 vials	2-8℃, 12 months shading light
Reagent 2	13.3 mmol/L D-Xylose Standard	1 mL × 1 vial	1 mL × 1 vial	2-8℃, 12 months
Reagent 3	Standard Diluent	10 mL × 1 vial	10 mL × 1 vial	2-8℃, 12 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Spectrophotometer, Micropipettor, Vortex mixer, Water bath

Reagents:

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

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of 1.33 mol/L standard solution：
Dilute 10 μL of 13.3 mmol/L D-Xylose standard with 90 μL of standard diluent. Mix well to dissolve. The 1.33 mol/L standard solution should be prepared on spot. Store at 2-8°C for 3 months protected from light.

Sample preparation

Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Urine: Collect fresh urine and centrifuge at 10000 g for 15 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection. If not detected on the same day, the urine can be stored at -80℃ for a month.

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.

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 phloroglucinol 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.

Calculation

The sample:

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

[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.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.05	1.20	3.50
%CV	2.5	2.0	2.1

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.05	1.20	3.50
%CV	4.3	4.8	4.4

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 103%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	0.1	1.5	3
Observed Conc. (mmol/L)	0.1	1.5	3.2
Recovery rate (%)	104	100	105

Sensitivity

The analytical sensitivity of the assay is 0.007 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix II Example Analysis

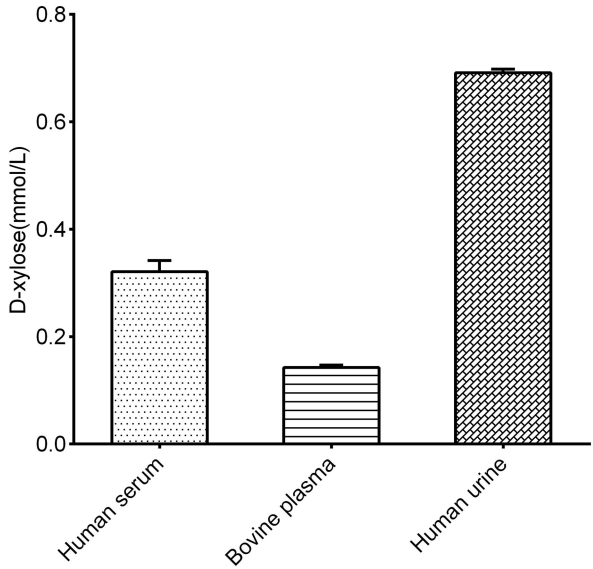
Example analysis：

For human serum, take 0.03 mL of human serum and 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

Detect human serum, bovine plasma, human urine (dilute for 10 times) according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.