D-XyloseMicroplate Assay Kit

User Manual

Catalog # CAK1238

(Version 2.2C)

Detection and Quantification ofD-XyloseContentin Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluidsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

In nature, D-xylose occurs mainly in the polysaccharide form as xylan, arabinoxylan, glucuronoarabinoxylan, xyloglucan and xylogalacturonan. Mixed linkage D-xylans are also found in certain seaweed species and asimilar polysaccharide is thought to make up the backbone of psyllium gum. In humans, D-xylose is used in an absorption test to help diagnose problems that prevent the small intestine from absorbing nutrients, vitamins and minerals in food. D-Xylose is normally easily absorbed by the intestine. When problems with absorption occur, D-xylose is not absorbed and blood and urine levels are low. A D-xylose test can help to determine the cause of a child's failure to gain weight, especially when the child seems to be eating enough food. If, in a polysaccharide, the ratio of D-xylose to other sugars etc. is known, then the amount of the polysaccharide can be quantified from this knowledge plus the determined concentration of D-xylose in an acid hydrolysate. Xylans are a major portion of the polysaccharides that could potentially be hydrolysed to fermentable sugar for biofuel production.

D-Xylose Microplate Assay Kit provides a convenient tool for sensitive detection of D-Xylose in a variety of samples. D-xylose is oxidised by NAD+ to D-xylonic acid in the presence of xylose dehydrogenase. D-Xyloseis measured by the increase in absorbance at 450 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Coenzyme	Powder x 1	-20 °C
Enzyme	0.1 ml x 1	4 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Coenzyme: add 1 mlReaction Buffer to dissolve before use.

Enzyme: add 1 mlReaction Buffer to dissolve before use.

Dye Reagent A: add 9 ml distilled water to dissolve before use, mix, store at 4°C.

Standard: add 1 ml distilled water to dissolve before use; then add 0.05 ml into 0.95 ml distilled water, mix, the concentration will be 1 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay buffer for 5 ×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 10000g 4°C for 15 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay buffer, centrifuged at 10000g 4°C for 15 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	60 µl	60 µl	60 µl
Sample	20 µl	--	--
Standard	--	20 µl	--
Assay Buffer	--	--	20 µl
Coenzyme	10 µl	10 µl	10 µl
Enzyme	10 µl	10 µl	10 µl
Mix, cover the plate adhesive strip, put the plate into the convection oven, incubate at 37 °C for 10 minutes.			
Dye Reagent A	90 µl	90 µl	90 µl
Dye Reagent B	10 µl	10 µl	10 µl
Mix, measured at 450 nmimmediately and recordthe absorbance.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the weight of sample

$$\text{D-Xylose (μmol/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$$

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

2. According to the volume of sample

$$\text{D-Xylose (μmol/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the concentration of standard, 1 mmol/L = 1μmol/ml;

W: the weight of sample, g;

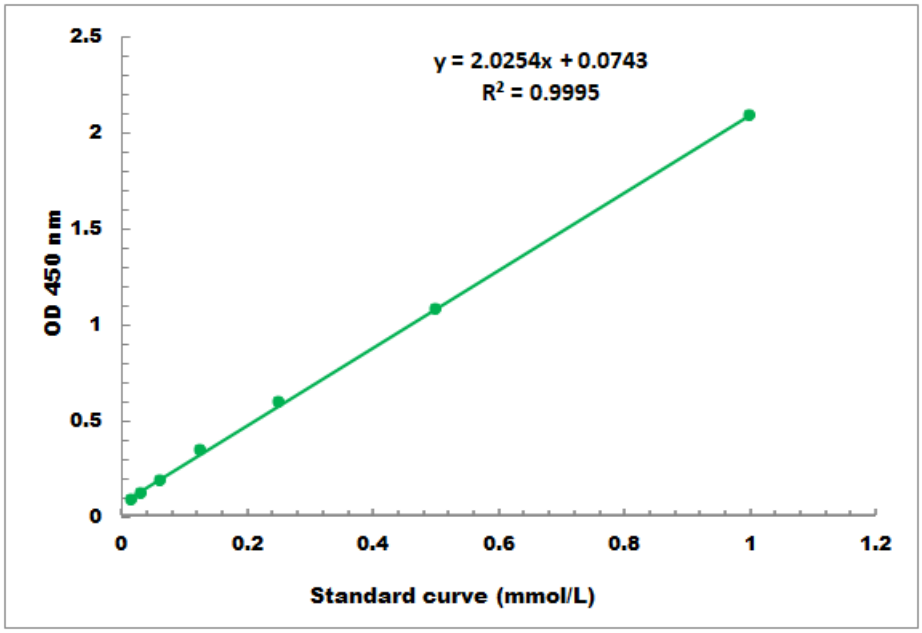
$V_{Standard}$: the volume of standard, 0.02 ml;

V_{Sample}: the volume of sample, 0.02 ml;

V_{Assay}: the volume of Assay Buffer, 1 ml.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.01 mmol/L - 1 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES