D-Xylose

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1238

(Version 2.3D)

Detection and Quantification of D-Xylose Content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluids Samples.

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 a similar 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 Colorimetric 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-xylose is 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	Powder x 1	-20 °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	

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. REAGENT PREPARATION

Coenzyme: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use, store at 4 °C for 1 month after reconstitution.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use, store at -80 °C for 1 month after reconstitution.

Dye Reagent A: Briefly centrifuge prior to opening. Add 9 ml distilled water to dissolve before use, mix, store at 4°C for 1 month after reconstitution.

Standard: Briefly centrifuge prior to opening. 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, store at 4 °C for 1 month after reconstitution. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 1/0.5/0.25/0.125/0.063/0.031/0.016/0.008 mmol/L.

V. SAMPLE PREPARATION

1. For plant tissue samples

Weigh 100 mg of material into a screw-cap tube. Add 5 mL of 1.3 M HCl to each tube and cap the tubes. Incubate the tubes at 100 °C for 1 h. Stir the tubes intermittently during the incubation. Cool the tubes to room temperature, add 5 mL of 1.3 M NaOH to neutralize the pH. Adjust the volume to 100 mL with distilled water centrifuge at 1,500 g for 10 min, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay 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.

3. For liquid samples

Detect directly.

VI. ASSAY PROCEDURE

Add following reagents into the 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 nm immediately and record the absorbance.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

** 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.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{Sample} - OD_{Blank}) - Intercept}{Slope} \times n \quad (mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the protein concentration of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times C_{Protein} \times V_{Sample}} \quad (\mu mol/mg)$$

2.2 According to the quantity of cells or bacteria

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay})} \quad (\mu mol/10^4)$$

2.3 According to the weight of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay})} \quad (\mu mol/g)$$

2.4 According to the volume of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample}} \quad (\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

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

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume of Assay Buffer, μl

$C_{Protein}$: the sample protein concentration, mg/ml

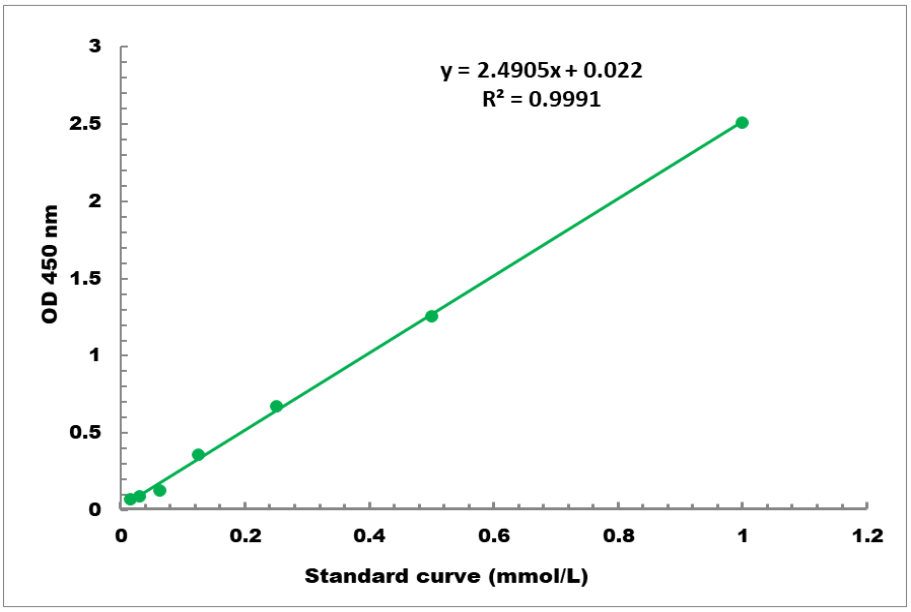
W: the weight of sample, g

V: the volume of sample in sample preparation, ml

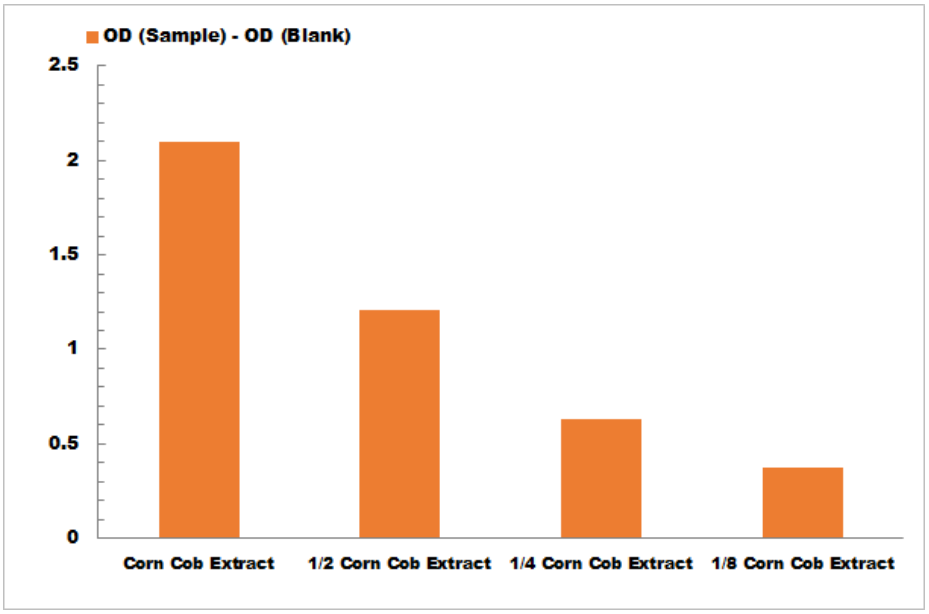
N: the quantity of cell or bacteria, 10^4

VIII. 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



Determination of D-xylose in Core Cob