D-Xylose Assay Kit

ARG83420 D-Xylose Assay Kit can be used to measure D-Xylose in urine, serum, plasma, tissue extracts, cell lysate, cell culture media and other biological fluids.

Catalog number: ARG83420

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION	Page
INTRODUCTION	3
PRINCIPLE OF THE ASSAY	3
MATERIALS PROVIDED & STORAGE INFORMATION	3
MATERIALS REQUIRED BUT NOT PROVIDED	4
TECHNICAL HINTS AND PRECAUTIONS	4
SAMPLE COLLECTION & STORAGE INFORMATION	4
REAGENT PREPARATION	5
ASSAY PROCEDURE	6
CALCULATION OF RESULTS	6
EXAMPLE OF TYPICAL RESULT	8

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INTRODUCTION

Xylose is a sugar first isolated from wood, and named for it. Xylose is classified as a monosaccharide of the aldopentose type, which means that it contains five carbon atoms and includes an aldehyde functional group.

PRINCIPLE OF THE ASSAY

The D-Xylose Assay Kit can measure D-Xylose in Urine, serum, plasma, tissue extracts, cell lysate, cell culture media and other biological fluids. D-xylose is oxidised by NAD+ to D-xylonic acid in the presence of xylose dehydrogenase. The increase in absorbance at 450 nm is directly proportional to reactants of D-Xylose.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4°C
Assay Buffer	4 X 30 ml (ready to use)	4°C
Reagent Dye A	1 vial (lyophilized)	4°C
Reagent Dye B	1 ml (ready to use)	4°C
Reaction Buffer	10 ml (ready to use)	4°C
Coenzyme	1 vial (lyophilized)	-20°C
Enzyme	0.1 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Coenzyme at -20°C, all other components store at 4°C.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell and bacteria- Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5 × 106 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.

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

*Note: liquid samples can detect directly.

REAGENT PREPARATION

- **Standard:** Add **1 ml** of **Distilled water** to dissolve standard, then add 0.05 ml into 0.95 ml **Distilled water**, the concentration will be 1 µmol /ml. Perform 2-fold serial dilution of the top standards to make the standard curve.
- **Reagent Dye A**: Add **9 ml** of **Distilled water** to dissolve Reagent Dye A before use.
- **Coenzyme**: Add **1 ml** of **Reaction Buffer** to dissolve Coenzyme before use.
- **Enzyme**: Add **1 ml** of **Reaction Buffer** to dissolve Enzyme before use.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add **60 µl Reaction Buffer** to each wells.
2. Sample wells: Add **20 µl Sample** into Sample wells.
3. Standard wells: Add **20 µl Standard** into Standard wells.
4. Add **10 µl Coenzyme** to each wells.
5. Add **10 µl Enzyme** to each wells.
6. Mix well. Incubate at **37°C** for **10 min**.
7. Add **90 µl Reagent Dye A Solution** to each wells.
8. Add **10 µl Reagent Dye B Solution** to each wells.
9. Read the OD at **450nm**.

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 well. Incubate at **37°C** for **10 min**			
Reagent Dye A	90 µl	90 µl	90 µl
Reagent Dye B	10 µl	10 µl	10 µl
Read the OD at **450nm**			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

2. Calculation:

A. Definition:

$C_{Standard}$: the standard concentration, 0.001 mmol /ml=1 µmol /ml;

W: the weight of sample, g;

V_{Sample}: the volume of reaction sample, 20 µl = 0.02 ml;

$V_{standard}$: the volume of standard, 20 µl = 0.02 ml;

V_{assay}: the volume of Assay Buffer, 1000 µl = 1 ml.

B. Formula:

a). According to the weight of sample

D-Xylose (µmol/g) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \times (W \times V_{Sample} / V_{assay})]$

$= (OD_{Sample} - OD_{blank}) / [(OD_{Standard} - OD_{Blank}) \times W]$

b). According to the volume of sample

D-Xylose (µmol/ml) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \times V_{Sample})]$

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

3. Detection range:

The detection range is from 0.01 µmol/ml - 1 µmol/ml.

4. If the samples have been diluted, the calculated activity must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and serial diluted standards are not necessary for this kit.