Acidic Xylanase Microplate Assay Kit

User Manual

Catalog # CAK1090

(Version 2.3D)

Detection and Quantification of Acidic Xylanase (ACX) Activity in Animal feeds, Enzyme preparations, Bread improver mixtures and other materials Samples.

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

I. INTRODUCTION

Xylanase (EC 3.2.1.8) is the name given to a class of enzymes which degrade the linear polysaccharide beta-1,4-xylan into xylose, thus breaking down hemicellulose, one of the major components of plant cell walls. As such, it plays a major role in micro-organisms thriving on plant sources for the degradation of plant matter into usable nutrients. Xylanases are produced by fungi, bacteria, yeast, marine algae, protozoans, snails, crustaceans, insect, seeds, etc., (mammals do not produce xylanases).

Acidic Xylanase Microplate Assay Kit provides a convenient tool for sensitive detection of Acidic Xylanase activity in a variety of samples. Xylan is broken down into xylose by xylanase. Xylose is oxidised by NAD+ to D-xylonic acid in the presence of xylose dehydrogenase. The xylanase activity 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
Substrate	Powder 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
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
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Note:

Substrate: add 10 ml Assay Buffer to dissolve before use, store at -20 °C for 1 month.

Coenzyme: add 1 ml Assay Buffer to dissolve before use, store at -80 °C for 1 month.

Enzyme: add 1 ml Assay Buffer to dissolve before use, store at -80 °C for 1 month.

Dye Reagent A: add 7 ml distilled water to dissolve before use, mix, store at 4°C for 1 month.

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, store at -20 °C for 1 month.

Positive Control: add 0.1 ml Assay Buffer before use, mix, store at -80 °C for 1 month.

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
8. Convection oven

IV. SAMPLE PREPARATION

1. For animal feeds, enzyme preparations, bread improver mixtures samples

Weigh out 0.1 g sample, homogenize with 1 ml Assay buffer, centrifuged at 8,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube for detection.

2. For liquid sample

Add 0.1 ml sample into 0.9 ml Assay buffer, centrifuged at 8,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube for detection.

V. ASSAY PROCEDURE

Add following reagents in the microcentrifuge tube:

Reagent	Sample	Control	Standard	Blank	Positive Control
Sample	10 µl	--	--	--	--
Distilled water	--	10 µl	--	--	--
Positive Control	--	--	--	--	10 µl
Substrate	90 µl	90 µl	--	--	90 µl
Standard	--	--	100 µl	--	--
Distilled water	--	--	--	100 µl	--
Coenzyme	10 µl	10 µl	10 µl	10 µl	10 µl
Enzyme	10 µl	10 µl	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	70 µl	70 µl	70 µl	70 µl	70 µl
Dye Reagent B	10 µl	10 µl	10 µl	10 µl	10 µl
Mix, incubate for 10 minutes, measured at 450 nm and record the absorbance.					

Note:

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

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

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

VI. CALCULATION

Unit Definition: One unit of Acidic Xylanase activity is defined as the enzyme generates 1 µmol of xylose per minute.

1. According to the weight of sample

$$ACX\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

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

2. According to the volume of sample

$$ACX\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times V / V_{Assay}) / T$$

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

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

W: the weight of sample, g;

V: the volume of sample, ml;

$V_{Standard}$: the volume of standard, 0.1 ml;

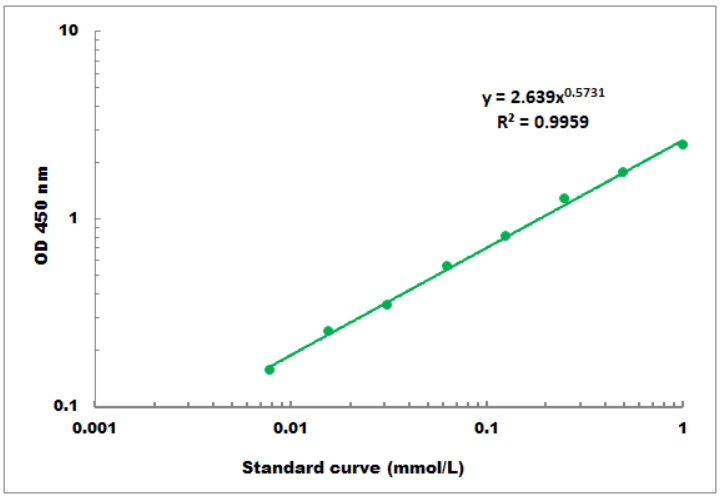
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer in sample preparation, 1 ml;

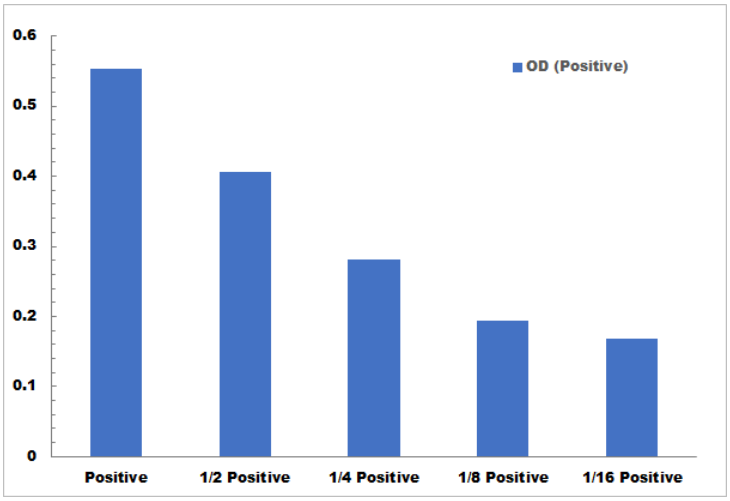
T: the reaction time, 10 minutes.

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



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

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

IX. NOTES