Acidic Xylanase Microplate Assay Kit

User Manual

Catalog # FTA0089

(Version 1.2C)

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).

The assay is initiated with the enzymatic hydrolysis of the xylan by acidic xylanase. The enzyme catalysed reaction products react with 3,5-dinitrosalicylic acid, and can be measured at a colorimetric readout at 540 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
Dye Reagent	10 ml x 1	4 °C, keep in dark
Standard (2.5 µmol/ml)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 10 ml Assay Buffer to dissolve before use, store at 4 °C.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 540 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
Sample	10 µl	--	--	--
Distilled water	--	10 µl	--	--
Substrate	90 µl	90 µl	--	--
Mix, put it in the oven, 50 °C for 10 minutes.				
Standard	--	--	100 µl	--
Distilled water	--	--	--	100 µl
Dye Reagent	100 µl	100 µl	100 µl	100 µl
Mix, put it into the convection oven, 90 °C for 10 minutes, when cold record absorbance measured at 540 nm.				

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.

VI. CALCULATION

Unit Definition: One unit of Acidic Xylanase activity is defined as the enzyme generates 1 μmol of reducing sugar per hour at 50 °C, pH4.8.

1. According to the weight of sample

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

$$= 2.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

2. According to the volume of sample

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

$$= 2.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V$$

$C_{Standard}$: the concentration of Standard, 2.5 μmol/ml;

W: the weight of sample, g;

V: the volume of sample, ml;

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

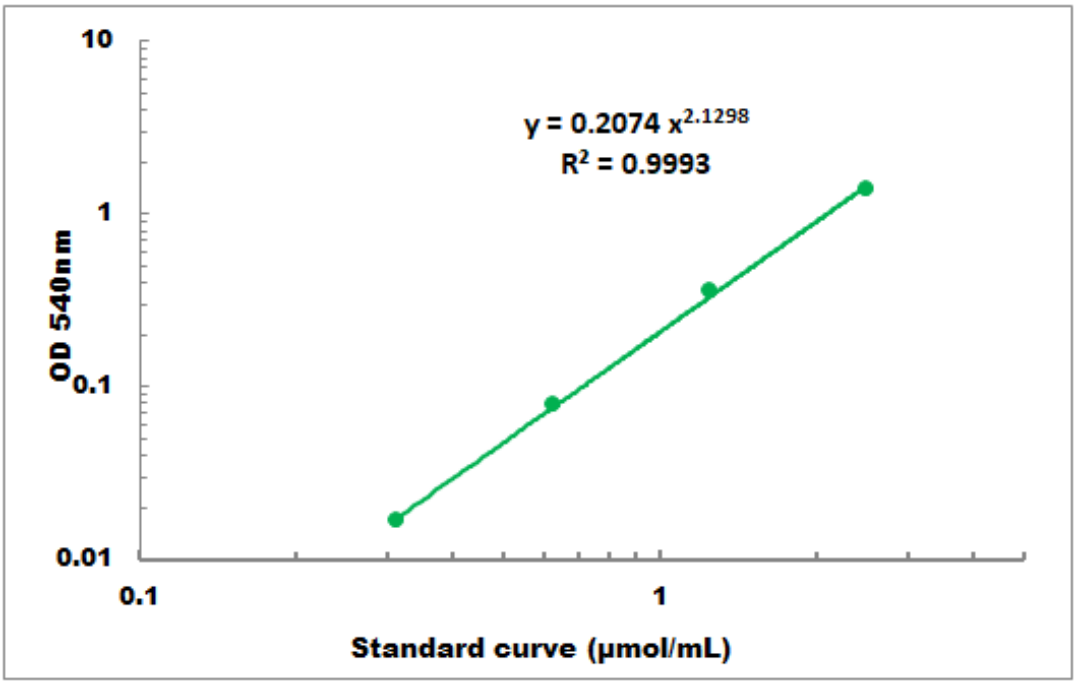
V_{Sample}: the volume of sample, 0.01 ml;

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: 0.25 µmol/mL - 2.5 µmol/mL

VIII. TECHNICAL SUPPORT

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

IX. NOTES