Neutral Xylanase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1089

(Version 2.5E)

Detection and Quantification ofNeutral Xylanase (NEX)Activityin Animal feeds, Enzyme preparations, Bread improver mixtures and other materialsSamples.

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).

Neutral Xylanase ActivityColorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of neutral xylanaseactivity 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 activityis measured by the increase in absorbance at 450 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Substrate	Powderx 1	4 °C
Coenzyme	Powder x 1	-20 °C
Enzyme	Powder 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	Powderx 1	-20 °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
8. Convection oven

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 20 mmol/L of standardstock solution, store at 4 °C for 3-4 monthsafter reconstitution.Then dilute to 1 mmol/Lstandard top solutionby adding 50 µlstock solution into 950 µldistilled water**.** Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 1/0.5/0.25/0.125/0.0625/0.0312/0.0156mmol/L.

Substrate: Add 10 mlAssay Buffer to dissolve before use. Store at 4 °C for 4-5 days or -20°C for 1 month after reconstitution.

Coenzyme: Briefly centrifuge prior to opening. Add 1 mlAssay Buffer to dissolve before use.Keep in dark and store at 4°C for 1 week or -20 °C for 2 months

Enzyme: Briefly centrifuge prior to opening. Add 1 mlAssay 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. Keep in dark and store at 4°C for 1 week or -20°C for 1 month.

Positive Control: Briefly centrifuge prior to opening. Add 0.1mlAssay Buffer before use, mix, store at -80°C for 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

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

Weighout 0.1 g sample, homogenize with 1 mlAssay 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 mlAssay Buffer, centrifuged at 8,000g 4°C for 20 minutes, take the supernatant into a new centrifuge tube for detection.

VI. ASSAY PROCEDURE

Add following reagents in the microcentrifuge tube:

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Sample	10 µl	--	--	--	--
Positive Control	--	--	--	--	10 µl
Substrate	90 µl	90 µl	--	--	90 µl
Standard	--	--	100 µl	--	--
Distilled water	--	10 µl	--	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 at 37 °C for 10 minutes,measured at 450 nm and recordthe absorbance.					

Note:

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

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of Neutral Xylanase activity is defined as the enzymegenerates 1μmol of xylose per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Control}) - \text{Intercept}}{\text{Slope}\times T} \times \frac{V_{Standard}}{V_{Sample}} \times n(U/mL)$$

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

2.1 According to the weight of sample

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

2.2 According to the volume of sample

$$\text{Activity}= \frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times V / (V + V_{Assay})\times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of total sample, g

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

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

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

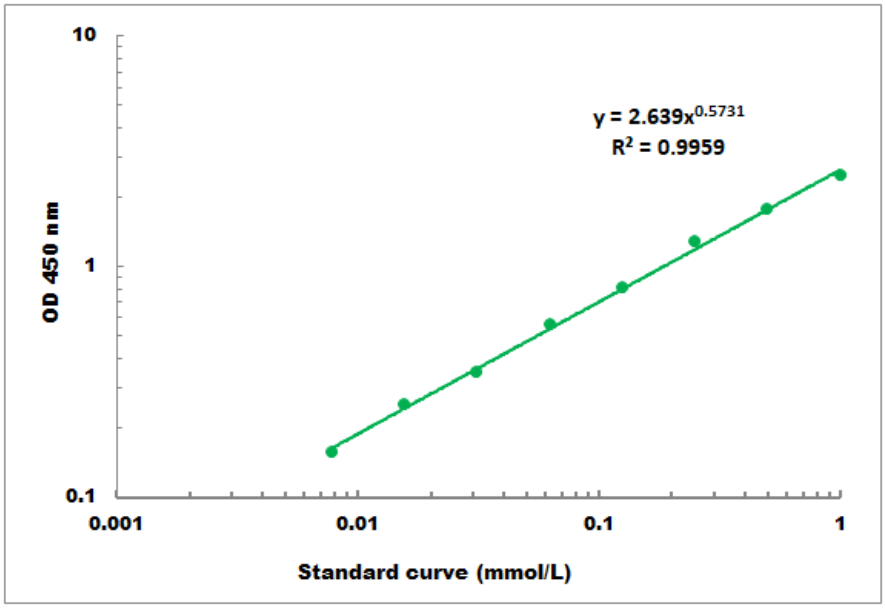
V_{Assay}: the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

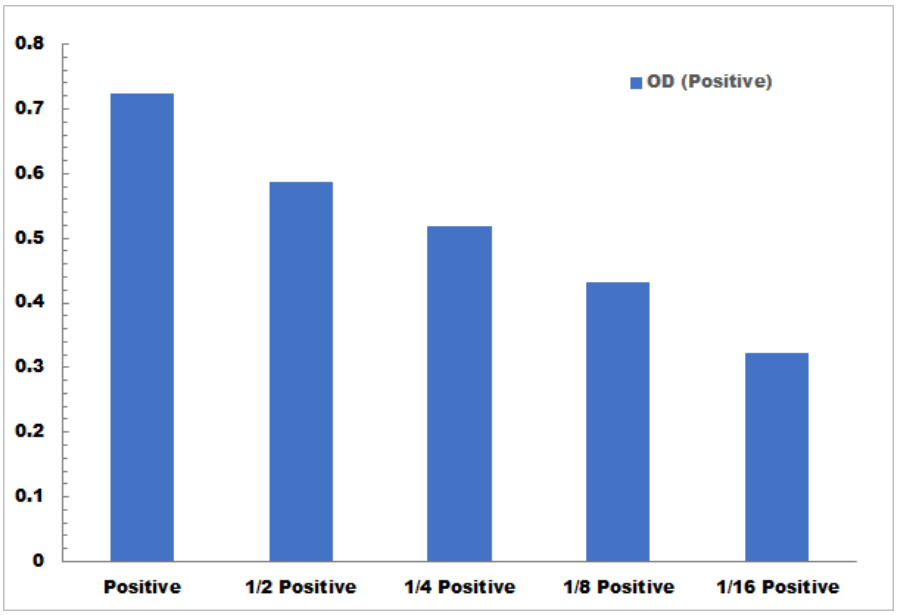
V: the volume of sample in sample preparation, mL

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



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