

XYLAZYME AX TABLETS

ASSAY PROTOCOL

T-XAX

10/22

For the assay of *endo*-1,4- β -XYLANASE

200 Tablets / 1000 Tablets



Play Training Video



INTRODUCTION:

Xylanase (*endo*-1,4- β -D-xylan xylanohydrolase; EC 3.2.1.8) finds widespread application in chicken feed supplements, in bread improver mixtures and in the enzymic bleaching of wood pulp (Nissen *et al.*, 1992¹). Historically, xylanase activity has been measured with the DNS reducing sugar assay (Bailey, 1988²) with purified xylan from oat spelts, larchwood or birchwood as substrate. However, these assays are limited by their lack of sensitivity and linearity, and the fact that they cannot be used to assay activity in materials containing high levels of reducing sugars, e.g. chicken feeds and bread improver mixtures.

In this assay protocol, the assay procedure for the measurement of xylanase using insoluble, dyed, crosslinked arabinoxylan - Xylazyme AX (**T-XAX**) is outlined. Other methods such as reducing sugar assay using Nelson Somogyi procedure³ and viscometry-based assays⁴ are also suitable for use in measuring xylanase activity. These assays can be performed using Neogen's Megazyme brand wheat arabinoxylan (**P-WAXYH** or **P-WAXYM**) (Please refer to the relevant product page for more information).

The use of the Xylazyme AX procedure is recommended over these other xylanase activity measurement methods due to its convenience, reliability, and accuracy.^{5,6} If a soluble, dyed, xylan substrate is preferred, Azo-Xylan (Birchwood) (Cat. no. **S-AXBP**) is the substrate of choice. It should be noted that the reproducibility of the **S-AXBP** substrate is superior to Xylazyme AX, but the sensitivity of the product is much lower.

SUBSTRATE:

Xylazyme AX is an azurine-crosslinked wheat arabinoxylan substrate. This substrate is prepared by dyeing and crosslinking highly purified wheat arabinoxylan to produce a material that hydrates in water but is water insoluble. Hydrolysis by *endo*-1,4- β -D-xylanase produces water soluble dyed fragments, and the rate of release of these (increase in absorbance at 590 nm) can be related directly to enzyme activity. The substrate is supplied commercially in a ready-to-use tablet form to aid usage.

PREPARATION OF BUFFERS (not supplied):

The most suitable buffer set to choose (**buffer B** or **buffer D**) depends on the enzyme activity profile of the user's xylanase.

(A) Concentrated Acetate Buffer [Sodium acetate (1 M, pH 4.7)]

Add 60.0 g of glacial acetic acid (1.05 g/mL) to 800 mL of distilled water. Adjust the pH of this solution to pH 4.7 by the addition of 4 M (16 g/100 mL) NaOH solution. Then adjust the volume to one litre. Store at room temperature.

(B) Acetate Extraction/Dilution Buffer [Sodium acetate (25 mM, pH 4.7)]

Add 25 mL of **concentrated acetate buffer** (A) to 950 mL of distilled water. Adjust

the pH of the solution to 4.7 by dropwise addition of 2 M HCl acid then bring the volume to one litre. Store at room temperature. This is **buffer B**.

(C) Concentrated Phosphate Buffer [Sodium Phosphate (0.5M, pH 6.0)]

Add 156 g of sodium dihydrogen orthophosphate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$) to 900 mL of distilled water. Adjust the pH to 6.0 with 4 M NaOH. Adjust the volume to 2 litres and (optionally) add 0.2 g of sodium azide. Store at 4°C.

(D) Phosphate Extraction/Dilution Buffer [Sodium Phosphate (25 mM, pH 6.0)]

Add 50 mL of **concentrated phosphate buffer (C)** to 940 mL of distilled water. Adjust the pH to 6.0 with 1 M HCl or 1 M NaOH. Add 0.2 g of sodium azide, dissolve and adjust the volume to one litre. The addition of sodium azide is optional. Store at 4°C. This is **Buffer D**.

IMPORTANT NOTE:

Do not add the sodium azide until the pH is adjusted. Acidification of sodium azide releases a poisonous gas. Sodium azide is a poisonous chemical and is added solely as a preservative against microbial infection. This chemical can be omitted from the buffers, but their long-term stability will be reduced.

STOPPING REAGENTS:

The most suitable stopping reagent for the user's assay is dependent on the enzyme's pH optima. See note 6.

A. Tris Buffer Salt Solution (2% w/v; pH 9.0)

Dissolve 20 g of tris buffer salt (Cat. no. **B-TRIS500**) in 1 L of distilled water. Store this solution at room temperature.

B. Tri-Sodium Phosphate [(Na₃PO₄·12H₂O); 2% w/v, pH 11] Dissolve 20 g of tri-sodium phosphate in 900 mL of distilled water. Adjust the pH to 11.0 with 4 M HCl and the volume to 1 L. Store this solution at room temperature.

NOTES:

1. Xylazyme AX tablets contain AZCL-wheat arabinoxylan, i.e. they are based on wheat arabinoxylan, the native substrate.
2. In the development of Xylazyme AX standard curves, the xylanase activity was first standardised in reducing sugar units using wheat arabinoxylan and the Somogyi reducing-sugar method.
3. In the assay format described here, a single blank is required for each set of determinations, and this is used to zero the spectrophotometer. The absorbance of the reaction solutions is measured against this blank.
4. The standard curve relating xylanase activity to absorbance at 590 nm is not always linear.
5. Stirring of the test tube on addition of the Xylazyme AX tablet to the enzyme

solution gives a slight (about 5%) increase in absorbance values, but it also gives less reproducible results. Consequently, tubes are not stirred after the addition of the substrate tablet.

6. AZCL-wheat arabinoxylan (the active ingredient in Xylazyme AX) has limited stability in solutions of high pH. Consequently, Tris buffer salt solution (pH ~ 9) is recommended. Where enzymes are assayed which are active at higher pH's (pH 9 - 10) (e.g. *Humicola insolens* xylanase in Novo Biofeed Plus) it is advisable to use 2% (w/v) tri-sodium phosphate (pH 11) as the stopping reagent. In these cases, the stopped reaction should be filtered within **5 min** of terminating the reaction.

ENZYME EXTRACTION AND DILUTION:

1. Using a positive displacement dispenser, add 1.0 mL of liquid enzyme sample to 99 mL of **buffer B** (pH 4.7) or **buffer D** (pH 6.0) and mix thoroughly. Alternatively, for powder enzyme samples, add 1.0 g to 100 mL of appropriate pH buffer and stir and extract the slurry over 15 min.
2. Add 0.5 mL of the solution obtained in step 1 to 4.5 mL of **buffer B or D** (i.e. dilute 10-fold). Repeat this process of dilution until a concentration suitable for assay is achieved. For example, certain industrial enzyme preparations require a dilution of the original preparation of 10,000-fold.
3. For chicken feeds, extraction of the enzyme is complicated by binding of enzyme to feed components (such as cellulose) and/ or the presence of xylanase inhibitor proteins. Consequently, extraction conditions may vary depending on the nature of the feed mixture and the particular xylanase employed. Please refer to the separate booklet (Xylanase in Animal Feeds; **K-XYLS**) for information on the measurement of xylanase in these materials.

EQUIPMENT (RECOMMENDED):

1. Glass test tubes (16 x 120 mm, ~ 16 mL capacity, round bottomed).
2. Micro-pipettors, 1,000 µL (e.g. Gilson Pipetman®).
3. Positive displacement pipettor, e.g. Eppendorf Multipette® with 5.0 mL Combitip® (to dispense 0.50 mL aliquots of enzyme concentrate).
4. Adjustable volume dispenser set at 10.0 mL (for Tris buffer salt or tri-sodium phosphate).
5. Bench centrifuge (required speed 3,000 rpm)(approx. 1,500 g).
6. Analytical and top-pan balances.
7. Vortex mixer.
8. Thermostated water bath set at 40.0.°
9. Stop clock.

10. Whatman No. 1 (9 cm) filter circles and filter funnels.

11. Spectrophotometer set at 590 nm.

ASSAY PROCEDURE:

1. Add 0.50 mL aliquots of suitably diluted enzyme preparation [in **buffer B** or **D** depending on the enzyme activity profile] to the bottom of glass test tubes (16 x 120 mm) and equilibrate at 40°C for 5 min.
2. Add a Xylazyme AX test tablet to initiate the reaction. The tablet rapidly hydrates. **Do not stir** the suspension.
3. Terminate the reaction **exactly 10 min** after the addition of the tablet by adding 10.0 mL of Tris buffer salt solution (2% w/v, pH ~ 9.0); or alternatively 10.0 mL of tri-sodium phosphate solution (2% w/v, pH 11.0; refer to Note 6, page 3). Stir the tube vigorously on a vortex mixer.
4. Leave the tubes to sit at **room temperature** for ~5 min and then stir the contents again. Filter the slurry through a Whatman No. 1 (9 cm) filter circle.
5. Measure the absorbance of the filtrate at 590 nm against a **substrate/enzyme** blank. The **substrate/enzyme** blank is prepared by adding 2% Tris buffer salt solution (or 2% tri-sodium phosphate solution) to the enzyme solution before the addition of the Xylazyme AX tablet. The slurry must be left at **room temperature**.

STANDARDISATION:

The standard curve relating the activity of xylanase from *A. niger* on Xylazyme AX tablets and on Wheat Arabinoxylan using the Somogyi reducing sugar method is shown in **Figure 1**.

CALCULATION OF ACTIVITY:

Xylanase activity in the sample assayed is determined by reference to the standard curve or regression equation to convert absorbance (590 nm) into milli-Units of activity per assay.

The regression equation varies slightly from batch to batch of Xylazyme AX tablets. To minimise this inconvenience, large batches of tablets are prepared.

Units/mL or gram of original preparation:

$$= \text{milli-Units (per assay, i.e. per 0.5 mL)} \times \frac{1}{1000} \times 100 \times 2 \times \text{Dilution}$$

where:

$$\frac{1}{1000} = \text{conversion from milli-Units to Units}$$

100 = initial extraction volume (i.e. 100 mL per g of solid).

2 = conversion from volume assayed (0.5 mL) to 1 mL of extract.

Dilution = further dilution of the initial extraction solution.

One Unit of enzyme activity is the amount of enzyme required to release one micromole of reducing sugar equivalents (as xylose by the Somogyi reducing-sugar method) from arabinoxylan per minute under standard assay conditions (40°C and pH 4.7 or 6.0).

NOTE: The regression equation shown in Fig. 1 should be used only over the absorbance range 0.2-2.0.

milli-Units/assay (i.e. 0.5 mL) = $41.7 \times \text{Abs.} + 2.4$; $R=0.99$

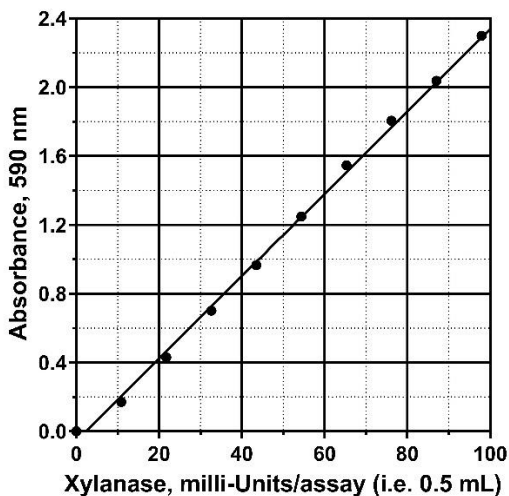


Figure 1. Pure *Aspergillus niger* xylanase standard curve on Xylazyme AX (Lot 120102) at pH 4.7. Reaction stopped with Tris buffer.

REFERENCES:

1. Nissen, A. M., Anker, L., Munk, N. & Krebs Lange, N. (1992). Xylanases for the pulp and paper industry. In *Xylans and Xylanases* (Visser, J., Beldman, G., Kusters-van Someren, M. A. & Voragen, A. G. J. Eds.), Elsevier Science Publishers, Amsterdam, pp. 325-338.
2. Bailey, M. J. (1988). A note on the use of dinitrosalicylic acid for determining the products of enzymatic reactions. *Applied Microbiology and Biotechnology*, **29**, 494-496.
3. Somogyi, M. (1960). Modifications of two methods for the assay of amylase. *Clinical Chem*, **6**, 23-35.
4. Buckee, G. K. & Baker, C. D. (1988). Collaborative trial on the determination of β -glucanases in malt by viscometric and dye-labelled methods. *J. Inst. Brew*, **96**, 387-390
5. McCleary, B. V. & Monaghan, D. (1999). New developments in the measurement of β -amylase, *endo*-protease, β -glucanase and β -xylanase. In *Proceedings of the Second European Symposium on enzymes in grain processing* (M. Tenkanen Ed.), VTT Information Service, pp 31-38.
6. McCleary, B. V. (2001). Analysis of feed enzymes. In "Enzymes in farm animal nutrition" (M. Bedford and G. Partridge), CAB International, pp 85-107.



Megazyme

**North America
Neogen Headquarters**

800.234.5333 (USA/Canada)
foodsafety@neogen.com
Neogen.com

**Europe, Middle East and Africa
Neogen Europe**

+ 44 (0) 1292 525 600
info_uk@neogeneurope.com
Neogen.com

**Mexico
Neogen Latinoamerica**

+52 (55) 5254-8235
informacion@neogenlac.com
Neogen.com

**Brazil
Neogen do Brasil**

+55 19 3935.3727
info@neogendobrasil.com.br
Neogen.com

**China
Neogen Bio-Scientific Technology**

+86 21 6271 7013
info@neogenchina.com.cn
www.neogenchina.com.cn

**India
Neogen Food and Animal Security**

+91 484 2306598, 2301582
info@neogenindia.com
www.neogenindia.com

Neogen

Megazyme Building 1,
Bray Business Park, Bray,
Co. Wicklow,
A98 YV29,
IRELAND.
+353 (1) 286 1220
Megazyme.com

Without guarantee

The information contained in this assay protocol is, to the best of our knowledge, true and accurate, but since the conditions of use are beyond our control, no warranty is given or is implied in respect of any recommendation or suggestions which may be made or that any use will not infringe any patents.