Megazyme

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endo-XYLANASE

ASSAY PROCEDURE

(XylX6 METHOD)

08/18

K-XylX6-1V

(100 Manual Assays per Kit) or
(200 Auto-Analyser Assays per Kit)

K-XylX6-2V

(200 Manual Assays per Kit) or
(400 Auto-Analyser Assays per Kit)

INTRODUCTION:

endo-Xylanase (*endo*-1,4-β-xylanase, EC 3.2.1.8) is an enzyme responsible for the hydrolytic degradation of xylans, a family of natural polysaccharides whose structure includes a main chain of β-1,4-linked D-xylopyranosyl residues which are decorated by residues of 4-*O*-methyl-D-glucuronic acid, D-glucuronic acid or L-arabinofuranose. *endo*-Xylanases are responsible for the hydrolysis of β-1,4 glycosidic bonds linking the xylopyranosyl residues present in the main chain. This enzyme is commercially important and finds applications in various industries. It is used in the feed industry to break down arabinoxylans in cereals thus improving the digestibility of cereal-based feeds for chickens and pigs, and the nutritional quality and digestibility of ruminant fodder. In addition, xylanases are widely employed in the baking, paper and pulp, detergents and biofuel industries, and more recently they have been applied to the production of xylo-oligosaccharides (XOS) and arabino-xylo-oligosaccharides (AXOS) for use as prebiotics in human nutrition. Numerous methods are available for the measurement of *endo*-β-xylanase, including those based on increase in reducing sugar levels on hydrolysis of commercially available native polysaccharides such as beechwood xylan (**P-XYLNBE**) and wheat arabinoxylan (**P-WAXYM**). With regard to this method, McCleary and McGeough have recently demonstrated that the commonly used DNS method grossly overestimates *endo*-xylanase activity and that if performing reducing sugar assays, the Nelson-Somogyi method must be employed.[1] Dyed soluble or insoluble (crosslinked) arabinoxylans[2] (**S-AWAXP**, **S-AWAXL, I-AZWAX**, **T-XAX**) can also be used for the convenient measurement of *endo*-xylanase, however these assay formats are poorly suited to automation due to the filtration/centrifugation step required.

To address this limitation, Megazyme has developed the novel soluble colourimetric reagent XylX6 described herein. Its defined chemical structure eliminates the possibility of batch to batch variability and the assay format allows for its application in automated analysis systems. The colourimetric substrate contained in the XylX6 assay kit (**K-XylX6**) is combined with a β-xylosidase which allows for the release of the colourimetric group 4-nitrophenol as outlined in Scheme 1, Appendix A, page 9. The rate of release of 4-nitrophenol relates directly to the rate of hydrolysis of XylX6 by the *endo*-xylanase being analysed. The XylX6 substrate exhibits the distinct advantage over commonly employed colourimetric oligosaccharide substrates in that the ketobutylidene acetal on the non-reducing terminal residue acts as a "blocking group" preventing hydrolysis by *exo*-acting enzymes including β-xylosidase and β-glucosidase which commonly occur in crude sample extracts. The reagent can be used

at temperatures up to 50°C and within the pH range of 4-7.5. Assay linearity, standard curves and reproducibility data are all shown in the appendices (Page 9 onwards).

ACCURACY:

Standard errors of less than 3% are readily achieved (see Tables 1 in Appendix D, page 14).

SPECIFICITY:

XylX6 is not hydrolysed by enzymes other than *endo*-xylanase (and *endo*-glucanase enzymes which have an *endo*-xylanase secondary activity), so it can be used for the specific assay of this enzyme in fermentation broths and industrial enzyme preparations.

SAFETY:

The general safety measures that apply to all chemical substances should be adhered to. For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website.

KITS:

Two kit sizes are available from Megazyme:
K-XylX6-1V is suitable for performing 100 assays in manual format or 200 assays in auto-analyser format.
K-XylX6-2V is suitable for performing 200 assays in manual format or 400 assays in auto-analyser format.

Bottle 1: (x1 or x2)

XylX6 reagent: lyophilised powder containing the XylX6 substrate and β-xylosidase plus sodium azide (0.09% w/w). Stable for > 4 years below -10°C.

Bottle 2:

Trichoderma sp. *endo*-1,4-β-xylanase standard solution (5 mL, ~ 2.86 U/mL; actual value stated on the vial label) in 50% aqueous glycerol plus sodium azide (0.02% w/v). Stable for > 4 years below -10°C.

PREPARATION OF REAGENT SOLUTIONS:

1. Add 5.0 mL of deionised water to the lyophilised powder contained in Bottle 1 (XylX6 reagent). To maximise the long term stability of this reagent, this solution should be aliquoted out into appropriately sized vials and stored below -10°C. This helps to minimise freeze/thaw cycles. Stable for up to 48 h at room temperature.
2. With a positive displacement pipette, dispense 0.5 mL of the

contents of bottle 2 to 9.5 mL of Buffer D and mix well. Store below -10°C when not in use. Once diluted, the standard is stable for at least 6 months below -10°C.

BUFFERS:

(A) Concentrated Acetate Buffer

(Sodium acetate buffer, 1 M, pH 4.5)

Add 60 g of glacial acetic acid (1.05 g/mL) to 800 mL of distilled water. Adjust the pH of this solution to 4.5 by the addition of 5 M (20 g/100 mL) NaOH solution. Adjust the volume to 1 L. Stable for > 2 years at room temperature.

(B) Acetate Extraction/Dilution Buffer

(Sodium acetate buffer, 100 mM, pH 4.5 containing 0.5 mg/mL BSA and 0.02% w/v sodium azide)

Add 100 mL of concentrated acetate buffer (A) to 850 mL of distilled water. Adjust the pH to 4.5 by dropwise addition of 2 M HCl or 2 M NaOH and adjust the volume to 1 L. Add 0.5 g of BSA and dissolve. Add 0.2 g of sodium azide and dissolve. Stable for > 1 year at 4°C.

(C) Concentrated Phosphate Buffer

(Sodium phosphate buffer, 0.5 M, pH 6.0)

Add 78 g of sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4.2H_2O$) to 800 mL of distilled water. Adjust the pH to 6.0 with 4 M NaOH and adjust the volume to 1 L. Stable for > 1 year at 4°C.

(D) Phosphate Extraction/Dilution Buffer

(Sodium phosphate buffer, 100 mM, pH 6.0 containing 0.5 mg/mL BSA and 0.02% w/v sodium azide)

Add 200 mL of concentrated phosphate buffer (C) to 750 mL of distilled water. Adjust the pH to 6.0 with 1 M HCl or 1 M NaOH and adjust the volume to 1 L. Add 0.5 g of BSA and dissolve. Add 0.2 g of sodium azide and dissolve. Stable for > 1 year at 4°C.

NOTE:

1. Do not add the sodium azide to buffers until they have been adjusted to pH 4.5 or above. Adding sodium azide to strong acidic solutions can result in the release of a poisonous gas.
2. If diluted buffer is prepared without adding sodium azide as a preservative, then it should be stored at 4°C and used within a week. Alternatively, this can be stabilised against microbial contamination by storing the buffer in a well-sealed Duran® bottle and adding 1 drop of toluene.

STOPPING REAGENT:

2% (w/v) Tris buffer (pH 10.0)

Dissolve 20 g of Tris buffer salt (**B-TRIS500**) in 900 mL of distilled water. Adjust the pH to 10.0 with 1 M NaOH and adjust the volume to 1 L. Stable for > 2 years at room temperature.

EQUIPMENT (RECOMMENDED):

1. Disposable plastic micro-cuvettes (1 cm light path, 1.5 mL), e.g. Plastibrand®, semi-micro, PMMA; Brand cat. no. 759115 (www.brand.de).
2. Micro-pipettors, e.g. Gilson Pipetman® (50 µL and 100 µL).
3. Positive displacement pipettor, e.g. Eppendorf Multipette®
   - 2.5 mL Combitip® (to dispense 0.05 mL substrate solutions).
   - 25 mL Combitip® (to dispense extraction buffer and 1.5 mL of Stopping Reagent).
4. Analytical balance.
5. Spectrophotometer set at 400 nm.
6. Vortex mixer (e.g. IKA® Yellowline Test Tube Shaker TTS2).
7. Stop clock.
8. Bench centrifuge or Whatman GF/A glass fibre filter paper circles.

CONTROLS, PRECAUTIONS AND BLANKS:

1. For each set of assays, a reagent blank value should be determined. To obtain this value, add 1.50 mL of Stopping Reagent to 0.05 mL of pre-equilibrated XylX6 reagent solution and then add 0.05 mL of diluted enzyme preparation. A single reagent blank determination is sufficient for each batch of assays.
2. XylX6 reagent blank absorbance values exceeding 0.2 are not acceptable. In these cases, the XylX6 reagent blank should be repeated and if the absorbance value still exceeds 0.2, the XylX6 reagent should be discarded since it is likely to have been contaminated with *endo*-xylanase.
3. If reaction values (after blank subtraction) exceed 1.0, then the enzyme preparation should be diluted in the appropriate buffer and re-assayed. Appropriate corrections to the calculations should then be made.
4. The relative hydrolytic activity of 8 *endo*-xylanases on XylX6 versus the native substrates, wheat arabinoxylan and beechwood xylan, is discussed in Appendix C, pages 10-13. It is recommended that the user generates their own standard curve

for their particular *endo*-xylanase to ensure the most accurate determination of enzyme activity.

A. MANUAL ASSAY PROCEDURE:

Enzyme Extraction and Dilution:

1. **Liquid enzyme preparations:** Add 1.0 mL of liquid enzyme preparation to 49 mL of the relevant Extraction/Dilution Buffer using a positive displacement dispenser (these solutions can be very viscous) and mix thoroughly. This is termed the **Original Extract**.
2. **Powder enzyme preparations:** Add 1.0 g of powder enzyme sample to 50 mL of the relevant Extraction/Dilution Buffer and gently stir the slurry over a period of approx. 15 min or until the sample is completely dispersed or dissolved. Clarify this solution by centrifugation (1,000 *g*, 10 min) or by filtration through Whatman No. 1 (9 cm) filter circles. This is termed the **Original Extract**.
3. Add 1.0 mL of the **Original Extract** to 9.0 mL of the relevant Extraction/Dilution Buffer (10-fold dilution) and mix thoroughly. This dilution process is repeated until a suitable concentration of *endo*-xylanase for assay is achieved.

Assay of *endo*-xylanase:

1. Dispense 0.05 mL aliquots of XylX6 reagent solution directly to the bottom of 13 mL glass tubes and pre-incubate the tubes at 40°C for approx. 3 min.
2. Pre-incubate a diluted *endo*-xylanase solution at 40°C for 3 min.
3. To each tube containing XylX6 solution, add 0.05 mL of *endo*-xylanase solution to the bottom of the tube, stir on a vortex mixer and incubate tubes at 40°C for exactly 10 min (from time of addition).
4. At the end of the 10 min incubation period, add 1.5 mL of Stopping Reagent and stir the tube contents.
5. Read the absorbance of the reaction solutions and the reagent blank at 400 nm against distilled water.

CALCULATION OF ACTIVITY:

Units of *endo*-xylanase per mL or g of enzyme solution being assayed:

One Unit of activity is defined as the amount of enzyme required to release one micromole of 4-nitrophenol from the XylX6 substrate in one minute under the defined assay conditions, and is termed a **XylX6 Unit.**

XylX6 Units/mL or g of original enzyme preparation:

$$= \frac{\Delta E_{400}}{\text{Incubation Time}} \times \frac{\text{Total Volume in Cell}}{\text{Aliquot Assayed}} \times \frac{1}{\varepsilon_{mM}} \times \frac{\text{Extraction Vol.}}{\text{Sample Vol.}} \times \text{Dilution}$$

where:

ΔA_{400}	= Absorbance (reaction) - Absorbance (blank)
Incubation Time	= 10 min
Total volume in cell	= 1.6 mL
Aliquot assayed	= 0.05 mL
ε_{mM} 4-Nitrophenol	= 18.1 (at 400 nm) in 2% Tris buffer (pH 10)
Extraction volume	= 50 mL per 1 mL or 1 g of original extract
Dilution	= Further dilution of the original extract (if required)

Thus:

XylX6 Units/mL or Units/g of original enzyme preparation:

$$= \frac{\Delta E_{400}}{10} \times \frac{1.6}{0.05} \times \frac{1}{18.1} \times \frac{50}{1} \times \text{Dilution}$$

$$= \Delta E_{400} \times 8.84 \times \text{Dilution}$$

Note that XylX6 Units can be converted to *endo*-xylanase Units on wheat arabinxylan or beechwood xylan using the standard curves provided as described in Appendix C, pages 10-13. Further information and individual standard curves for 8 *endo*-xylanases (4 x GH10 and 4 x GH11) are provided in the Supporting Information file located in the documents tab on the **K-XylX6** product page.

B. AUTOMATED ASSAY PROCEDURE:

EQUIPMENT (RECOMMENDED):

1. ChemWell®-T auto-analyser fitted with a 405 nm filter.
2. Polypropylene tubes (13 mL capacity).
3. Pipettors, 1 mL (e.g. Gilson Pipetman®) to dispense enzyme extract.
4. Adjustable-volume dispenser:
   - 0-10 mL (for Extraction Buffer).
5. Top-pan balance.
6 Vortex mixer (optional).
7. Stop Clock.
8. Microfuge or Whatman GF/A glass fibre filter paper circles (9 cm diameter).

PREPARATION OF REAGENTS:

All reagents are prepared as described above for the manual assay procedure with the exception of the stopping reagent, the preparation of which is described below.

PREPARATION OF STOPPING REAGENT:

500 mM Sodium Carbonate, pH 11.0.

Dissolve 53 g of sodium carbonate (anhydrous) in 1 L of distilled water and adjust the pH to approx. 11.0. Store in a sealed bottle. Stable for ~ 3 months at room temperature.

ENZYME EXTRACTION AND ASSAY:

1. The **Original Extract** is prepared as described above for the manual assay procedure.
2. Perform the assay using the **K-XylX6** ChemWell®-T assay files.

Automated Assay Parameters:

Assay volumes:	XylX6 Reagent:	0.025 mL
	Sample (extract):	0.025 mL
	Stopping Reagent:	0.300 mL
Reaction time:	10 min at 37°C	
Wavelength:	405 nm	
Assay type:	Stopped reaction	
Reaction direction:	Increase	

CALCULATION OF ACTIVITY (Automated Assay Procedure):

One Unit of activity is defined as the amount of enzyme, in the presence of excess thermostable β-xylosidase, required to release one micromole of 4-nitrophenol from XylX6 in one minute under the defined assay conditions, and is termed a **XylX6 Unit.**

XylX6 Units/mL or Units/g of original enzyme preparation:

$$= \frac{\Delta E_{405}}{\text{Incubation Time}} \times \frac{\text{Total Volume in Cell}}{\text{Aliquot Assayed}} \times \frac{1}{\varepsilon_{mM}} \times \frac{\text{Extraction Vol.}}{\text{Sample Vol.}} \times \text{Dilution}$$

where:

ΔA_{405} = Absorbance (reaction) - Absorbance (blank)

Incubation Time = 10 min

Total Volume in Cell = 0.350 mL

Aliquot Assayed = 0.025 mL

Apparent ε_{mM} 4-Nitrophenol = 12.456 (at 405 nm, recorded on a ChemWell®-T system in 500 mM sodium carbonate, pH 11)

Dilution = Dilution of the extract including the original extraction (e.g. 50 mL per 1 mL or 1 g of *endo*-1,4-β-xylanase)

Thus:

XylX6 Units/mL or g of original enzyme preparation =

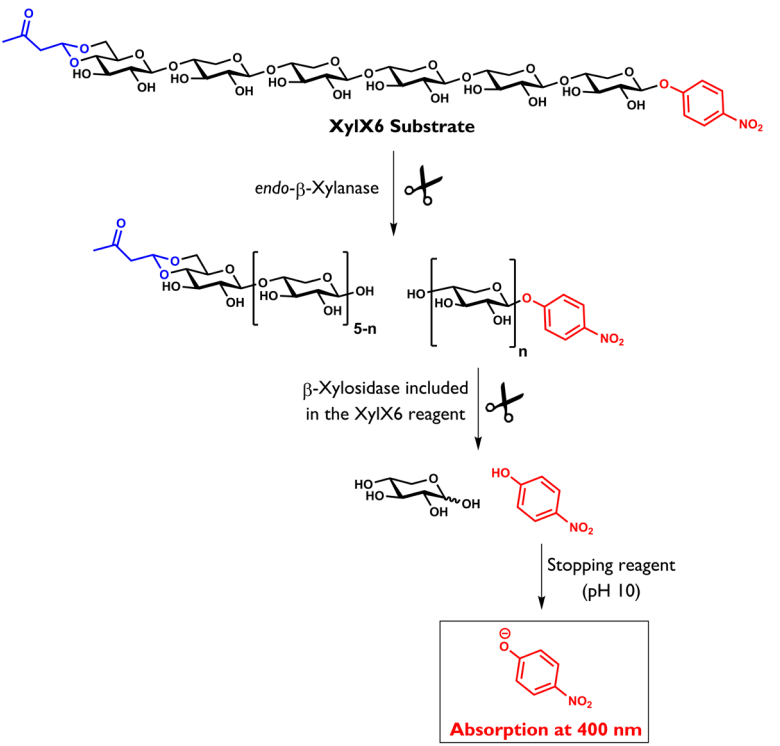
$$= \frac{\Delta E_{405}}{10} \times \frac{0.350}{0.025} \times \frac{1}{12.456} \times \text{Dilution}$$

$$= \Delta E_{405} \times 0.1124 \times \text{Dilution}$$

APPENDIX:

A. Principle of the assay

The XylX6 reagent contains two components: **1)** the XylX6 colourimetric substrate and **2)** β-xylosidase. The ketone blocking group at the non-reducing end prevents any hydrolytic action by the β-xylosidase on the XylX6 substrate. Incubation with an *endo*-xylanase generates a non-blocked colourimetric oligosaccharide that is rapidly hydrolysed by the ancillary β-xylosidase to quantitatively release the colourimetric group, 4-nitrophenol. The reaction is terminated and the phenolate colour is developed on addition of Tris buffer solution (pH 10.0). The absorption of the colourimetric group released is measured at 400 nm. The rate of formation of 4-nitrophenol is therefore directly related to the hydrolysis of the XylX6 substrate by the *endo*-xylanase.



Scheme 1. Rationale of the XylX6 *endo*-xylanase assay procedure.

B. Linearity

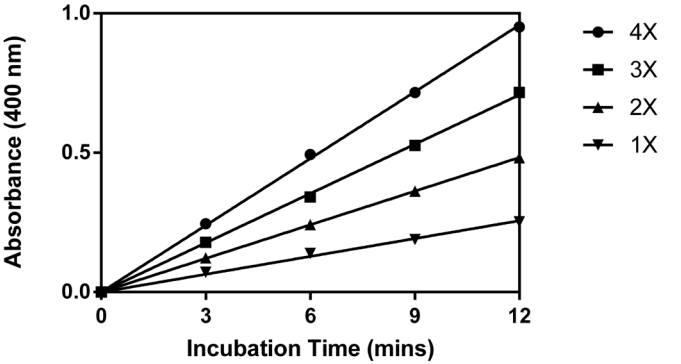


Figure 1. Linearity of XylX6 assay with *Trichoderma longibrachiatum* xylanase (**E-XYTR3**) in sodium acetate buffer (pH 4.5). Reaction was terminated at 3 min intervals by adding 2% Tris buffer solution (1.5 mL, pH 10).

C. Relationship between *endo*-xylanase hydrolysis of XylX6 and the native substrates, wheat arabinoxylan and beechwood xylan

endo-1,4-β-Xylanases from different organisms vary in their ability to hydrolyse different substrates. GH10 Xylanases display much higher activity on unsubstituted β-1,4-xylan and β-1,4-xylooligosaccharides than on arabinoxylan due to the highly substituted structure of the latter. Accordingly, GH10 xylanases exhibit higher activity on XylX6 (unsubstituted) followed by beechwood xylan (**P-XYLNBE**) (partially substituted) and wheat arabinoxylan (**P-WAXYM**) (highly substituted) in that order. Broadly speaking, GH11 xylanases (from fungal sources) do not exhibit large differences in their specific activities across these 3 substrates. Figures 2, 3 and 4 compare the relative activities of 8 xylanases, including 4 GH10s and 4 GH11s, across the 3 main substrates of interest. Equations allowing for the conversion of activities between these substrates for each enzyme are shown below each graph.

Note that the individual standard curves for each enzyme that relate the absorbance obtained at 400 nm using the XylX6 assay to *endo*-xylanase activity on the native substrates, beechwood xylan (**P-XYLNBE**) and wheat arabinoxylan (**P-WAXYM**), can be found in the supporting information file which can be downloaded from the **XylX6 product page** on www.megazyme.com.

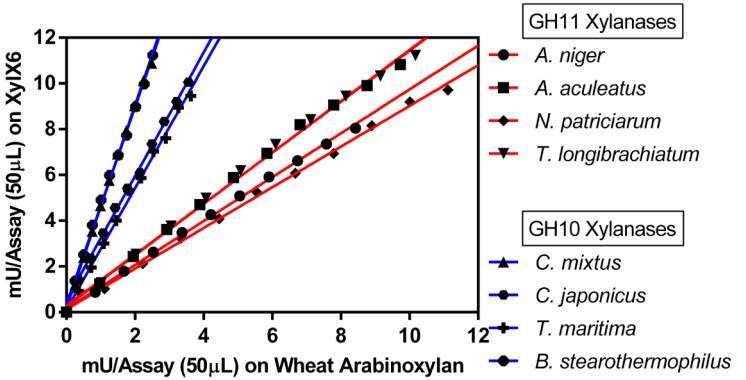


Figure 2. The relative hydrolytic activity of 8 xylanases on XylX6 and wheat arabinoxylan (**P-WAXYM**) is shown.

Conversions between *endo*-xylanase activity on XylX6 and wheat arabinoxylan (**P-WAXYM**) are shown below:

***Trichoderma longibrachiatum* endo-1,4-β-xylanase:**
Units on wheat arabinoxylan = 0.87 x XylX6 Units

***Aspergillus niger* endo-1,4-β-xylanase:**
Units on wheat arabinoxylan = 1.02 x XylX6 Units

***Neocallimastix patriciarum* endo-1,4-β-xylanase:**
Units on wheat arabinoxylan = 1.10 x XylX6 Units

***Aspergillus aculeatus* endo-1,4-β-xylanase:**
Units on wheat arabinoxylan = 0.86 x XylX6 Units

***Cellvibrio mixtus* endo-1,4-β-xylanase:**
Units on wheat arabinoxylan = 0.24 x XylX6 Units

***Cellvibrio japonicus* endo-1,4-β-xylanase:**
Units on wheat arabinoxylan = 0.34 x XylX6 Units

***Thermotoga maritima* endo-1,4-β-xylanase:**
Units on wheat arabinoxylan = 0.37 x XylX6 Units

***Bacillus stearothermophilus* endo-1,4-β-xylanase:**
Units on wheat arabinoxylan = 0.22 x XylX6 Units

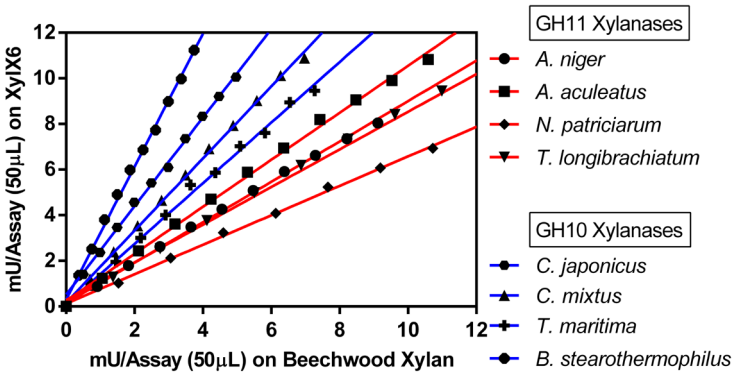


Figure 3. The relative hydrolytic activity of 8 xylanases on XylX6 and beechwood xylan (**P-XYLNBE**) is shown.

Conversions between *endo*-xylanase activity on XylX6 and beechwood xylan (**P-XYLNBE**) are shown below:

***Trichoderma longibrachiatum endo*-1,4-β-xylanase:**
Units on beechwood xylan = 1.17 x XylX6 Units

***Aspergillus niger endo*-1,4-β-xylanase:**
Units on beechwood xylan = 1.10 x XylX6 Units

***Neocallimastix patriciarum endo*-1,4-β-xylanase:**
Units on beechwood xylan = 1.52 x XylX6 Units

***Aspergillus aculeatus endo*-1,4-β-xylanase:**
Units on beechwood xylan = 0.94 x XylX6 Units

***Cellvibrio mixtus endo*-1,4-β-xylanase:**
Units on beechwood xylan = 0.66 x XylX6 Units

***Cellvibrio japonicus endo*-1,4-β-xylanase:**
Units on beechwood xylan = 0.48 x XylX6 Units

***Thermotoga maritima endo*-1,4-β-xylanase:**
Units on beechwood xylan = 0.74 x XylX6 Units

***Bacillus stearothermophilus endo*-1,4-β-xylanase:**
Units on beechwood xylan = 0.33 x XylX6 Units

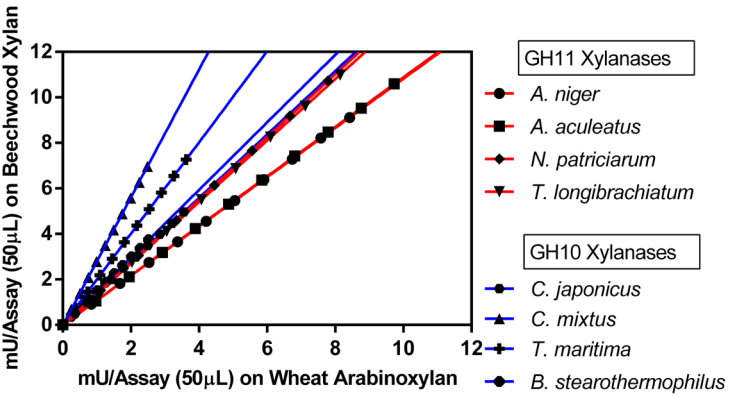


Figure 4. The relative hydrolytic activity of 8 xylanases on wheat arabinoxylan (**P-WAXYM**) and beechwood xylan (**P-XYLNBE**) is shown.

Conversions between *endo*-xylanase activity on wheat arabinoxylan (**P-WAXYM**) and beechwood xylan (**P-XYLNBE**) are shown below:

***Trichoderma longibrachiatum endo*-1,4-β-xylanase:**
Units on beechwood xylan = 1.35 x Units on wheat arabinoxylan

***Aspergillus niger endo*-1,4-β-xylanase:**
Units on beechwood xylan = 1.08 x Units on wheat arabinoxylan

***Neocallimastix patriciarum endo*-1,4-β-xylanase:**
Units on beechwood xylan = 1.38 x Units on wheat arabinoxylan

***Aspergillus aculeatus endo*-1,4-β-xylanase:**
Units on beechwood xylan = 1.09 x Units on wheat arabinoxylan

***Cellvibrio mixtus endo*-1,4-β-xylanase:**
Units on beechwood xylan = 2.81 x Units on wheat arabinoxylan

***Cellvibrio japonicus endo*-1,4-β-xylanase:**
Units on beechwood xylan = 1.40 x Units on wheat arabinoxylan

***Thermotoga maritima endo*-1,4-β-xylanase:**
Units on beechwood xylan = 2.01 x Units on wheat arabinoxylan

***Bacillus stearothermophilus endo*-1,4-β-xylanase:**
Units on beechwood xylan = 1.49 x Units on wheat arabinoxylan

D. Repeatability and Reproducibility

The repeatability and reproducibility of the assay was determined by having two analysts perform a series of assays on three different samples ranging in activity from **60-161 XylX6 mU/mL** over two consecutive days. The results are outlined in Table 1.

Table 1.

	ΔA obtained for *Trichoderma longibrachiatum* xylanase (E-XYTR3) in sodium acetate buffer (pH 4.5)		
Analysis	**161 mU/mL**	**121 mU/mL**	**60 mU/mL**
Day 1 A(i)	0.982	0.795	0.423
Day 1 A(ii)	0.980	0.794	0.433
Day 2 A(i)	0.993	0.807	0.437
Day 2 A(ii)	0.993	0.811	0.438
Day 1 B(i)	1.016	0.790	0.413
Day 1 B(ii)	1.028	0.764	0.413
Day 2 B(i)	1.055	0.784	0.419
Day 2 B(ii)	1.040	0.803	0.413
Standard Dev	**0.028**	**0.015**	**0.011**
% CV	**2.8**	**1.9**	**2.6**

Note: A=Analyst 1, B=Analyst 2, (i)=Extract 1, (ii)=Extract 2

Note that the Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as 3 x σ of the blank sample solution absorbance and 10 x σ of the blank sample solution absorbance, respectively, using absorbance values from 10 replicates.

LOD = 5.3 x 10^{-4} U/mL (corresponding to an absorbance of 0.003)
LOQ = 1.9 x 10^{-3} U/mL (corresponding to an absorbance of 0.011)

E. Standard curve for automated assay on ChemWell®-T Auto-Analyser.

All prior appendices apply to the manual assay format. The ChemWell®-T auto-analyser automatically converts the absorbance obtained in an assay into the *endo*-xylanase activity measurement (in XylX6 units) in the sample solution. Shown below is the calibration curve for automated XylX6 assay format on a ChemWell®-T auto-analyser.

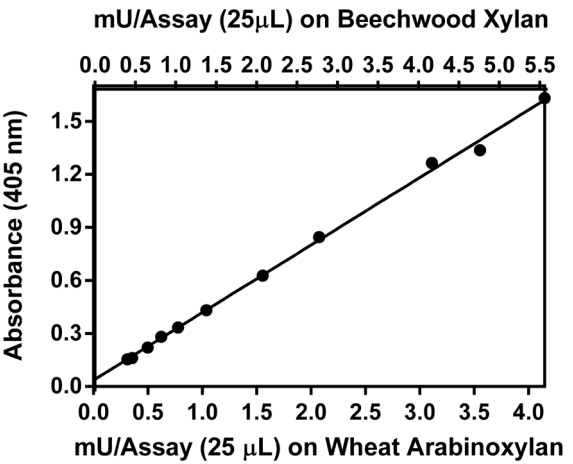


Figure 5. Standard curve relating the activity of *Trichoderma longibrachiatum endo*-1,4-β-xylanase (**E-XYTR3**) on both wheat arabinoxylan (medium viscosity) (**P-WAXYM**) and beechwood xylan (**P-XYLNBE**) to absorbance increase at 405 nm on hydrolysis of XylX6 obtained using a ChemWell®-T auto-analyser system under the recommended automated assay conditions.

REFERENCES:

1. McCleary, B.V. & McGeough, P. (2015). A Comparison of Polysaccharide Substrates and Reducing Sugar Methods for the Measurement of *endo*-1,4-β-Xylanase. *Applied Biochemistry Biotechnology,* 177, 1152–1163.

2. Somogyi, M. (1952). Note on sugar determination. *Journal of Biological Chemistry,* 195, 19-23.

3. McCleary, B.V. (1991). Measurement of Polysaccharide Degrading Enzymes using Chromogenic and Colorimetric Substrates. *Chemistry in Australia*, September, 398-401.

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