endo-XYLANASE (K-XylX6)
Procedure for ChemWell®-T Auto-Analyser

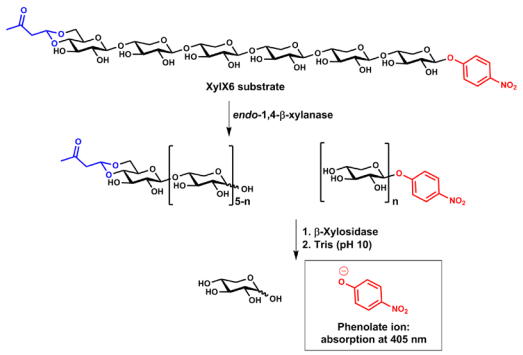
Requirements:

- *endo*-Xylanase Assay Kit **(K-XylX6-2V / K-XylX6-1V)** (provides ~ 400 assays or ~ 200 assays).
- **K-XylX6** ChemWell®-T assay file.
- Use in association with the *endo*-Xylanase Assay Kit (XylX6 Method) **(K-XylX6-2V or K-XylX6-1V)** product data booklet.

Use:

A specific and sensitive colourimetric method for the determination of *endo*-1,4-β-xylanase.

Assay Principle:



Procedure:

Prepare the assay reagents and use with the **K-XylX6** ChemWell®-T assay file.

Assay Kit Components:

Bottle 1: XylX6 reagent: lyophilized powder containing the XylX6 substrate and β-xylosidase plus sodium azide (0.09% w/w).
Stable for > 4 years at -20ºC.

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 at -20°C.

Preparation of Kit Components:

1. Add 5.0 mL of deionised water to the lyophilized powder contained in Bottle I (XylX6 reagent). Store at -20ºC when not in use. 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 at -20ºC when not in use. Once diluted, the standard is stable for at least 6 months at -20ºC.

Preparation of Reagents Not Supplied:

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.

(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 4ºC.

(C) Concentrated Phosphate Buffer

(Sodium phosphate buffer, 0.5 M, pH 6.0)

Add 78 g of sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$) 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 4ºC.

(D) Phosphate Extraction/Dilution Buffer

(Sodium phosphate buffer, 100 mM, pH 6.0 containing 0.5 mg/mL BSA and 0.02% 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 4ºC.

Extraction and Assay of Milled Grain Samples:

1. Add 1.0 mL of **liquid enzyme** preparation to 49 mL of Extraction/Dilution **Buffer B** (pH 4.5) **or Buffer D** (pH 6.0) using a positive displacement dispenser (these solutions can be very viscous) and mix thoroughly. **This is termed the Original Extract**.

   Alternatively:

   Add 1.0 g of **powder enzyme** sample to 50 mL of **Buffer B** (pH 4.5) **or Buffer D** (pH 6.0) 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 x *g*, 10 min) or by filtration through Whatman No. 1 (9 cm) filter circles. **This is termed the Original Extract**.

2. If further dilutions are required, add 1.0 mL of the **Original Extract** to 9.0 mL of **Buffer B** (pH 4.5) **or Buffer D** (pH 6.0) (10-fold dilution) and mix thoroughly. This process of dilution should be repeated until a suitable concentration of cellulase for assay is achieved.
3. Perform the assay using the **K-XylX6** ChemWell®-T assay file.

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Procedure for ChemWell®-T Auto-Analyser

Assay Parameters:

Assay volumes:

	XylX6 Reagent:	0.025 mL
	Sample:	0.025 mL
	Stopping Reagent:	0.300 mL

Reaction time: 10 min at 37ºC
Wavelength: 405 nm
Assay type: Stopped reaction
Reaction direction: Increase

Calculation of Activity:

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

XylX6 Units/mL or g of original enzyme preparation:

$$= \frac{\Delta E_{405}}{\text{Incubation Time}} \times \frac{\text{Total Volume in Cell}}{\text{Aliquot Assayed}} \times \frac{1}{\varepsilon_{mM}} \times D$$

where:

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

Incubation Time = 10 min

Total Volume in Cell = 0.350 mL

Aliquot Assayed = 0.125 mL

ε_{mM} of *p*-nitrophenol (at 405 nm) in sodium carbonate, pH 11 = 12.456

D = 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:

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

$$= \Delta E_{405} \times 0.1124 \times D$$

NOTE:
The absorption coefficient (ε_{mM}) of 12.456 was experimentally determined under the conditions of the automated *endo*-1,4-β-xylanase assay (XylX6 method) using a ChemWell®-T auto-analyser.

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