Megazyme

ASSAY OF

endo-1,4-ß-Xylanase

using

AZO-XYLAN (BIRCHWOOD)

S-AXBP
S-AXBL 10/07

Megazyme

PRINCIPLE:

This assay procedure is specific for *endo*-1,4-ß-D-xylanase activity. On incubation of Azo-Xylan (birchwood) with *endo*-xylanase, the substrate is depolymerised to produce low-molecular weight dyed fragments which remain in solution on addition of ethanol to the reaction mixture. High-molecular weight material is removed by centrifugation, and the colour of the supernatant is measured. *endo*-Xylanase in the sample solution is determined by reference to a standard curve.

SUBSTRATE:

Birchwood xylan is first purified (to remove starch) and then it is dyed with Remazolbrilliant Blue R to an extent of approx. one dye molecule per 30 sugar residues.

DISSOLUTION:

Add 1 g of powdered substrate to 80 mL of boiling and vigorously stirring water on a hot-plate stirrer. Turn the heat off and continue stirring until the polysaccharide is completely dissolved (about 20 min). Adjust the volume to 100 mL and add 0.02 g of sodium azide and dissolve. Store this solution at 4°C between use. Under these conditions the solution is stable for 12 months if contamination with enzyme is avoided. Shake the solution container before removing aliquots for assays. Because the solution is viscous, it should preferably be dispensed with a positive displacement dispenser (e.g. Eppendorf Multipette® with a 5.0 mL Combitip).

PRECIPITANT SOLUTION:

Industrial methylated spirits (IMS; 95% v/v) or ethanol (95% v/v).

BUFFER SOLUTIONS:

1. Sodium acetate buffer, 100 mM, pH 4.5

Add 6.0 g of glacial acetic acid (1.05 g/mL) to 800 mL of distilled water. Adjust the pH to 4.5 with 5 M (20 g/100 mL) sodium hydroxide solution. Adjust the volume to 1 litre. Stable for approx. 4 weeks at 4°C.

2. Sodium phosphate buffer, 100 mM, pH 6.0

Add 8.9 g of di-sodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$) to 450 mL of distilled water and dissolve. Adjust the pH to 6.0 with 1 M hydrochloric acid. Adjust the volume to 500 mL and add 0.1 g of sodium azide as a preservative. Stable for approx. 4 weeks at 4°C.

ENZYME EXTRACTION AND DILUTION:

Using a positive displacement dispenser, transfer 1.0 mL of liquid enzyme preparation to 49 mL of buffer 1 (100 mM sodium acetate buffer, pH 4.5) or buffer 2 (100 mM sodium phosphate buffer, pH 6.0) and mix thoroughly. This is termed the **Original Extract**. Dilute this solution 10-fold by transferring 1.0 mL of diluted enzyme to 9.0 mL of either buffer 1 or buffer 2. Repeat this process until a dilution of enzyme suitable for assay is obtained.

For powdered enzyme preparations, add 1.0 g of material to 50 mL of buffer 1 or buffer 2 and gently stir the slurry for 15 min, or until the sample is completely dispersed or dissolved. Clarify this solution (the **Original Extract**) by centrifugation at 1,000 *g* for 10 min, or by filtration through Whatman No. 1 (9 cm) filter circles. Dilute this solution as for the liquid enzyme preparations.

ASSAY PROCEDURE:

Add 0.5 mL of buffered enzyme preparation (pre-equilibrated to 40°C) to 0.5 mL of pre-equilibrated substrate solution (1% w/v Azo-Xylan birchwood) with thorough mixing on a vortex stirrer. Immediately return the mixture to the water bath and incubate at 40°C for exactly 10 min from the time of addition of the enzyme solution. Terminate the reaction by adding 2.5 mL of ethanol (95% v/v) with vigorous stirring on a vortex mixer to the reaction solution. This will precipitate high-molecular weight, non-hydrolysed substrate. Store the incubation tubes at room temperature for 5 min and stir them again. Centrifuge the tubes at 1,000 g (approx. 3,000 rpm for 10 min). Pour the supernatant solution directly into a spectrophotometer cuvette and measure the absorbance of the blank and reaction solutions at 590 nm against water. Determine the activity by reference to a standard curve. Alternatively, enter the absorbance values into the appropriate MegaCalc™ available from the Megazyme website (www.megazyme.com).

Prepare a reaction blank by adding 2.5 mL of ethanol (or IMS) to 0.5 mL of the substrate solution (1% w/v) with vigorous stirring. Immediately add 0.5 mL of the enzyme solution and stir the mixture vigorously for 10 sec. Because the diluted enzyme preparations are essentially colourless, a single blank only is required with each set of determinations. Typically, blank absorbance values at 590 nm are ~ 0.07.

A standard curve for *A. niger endo*-β-xylanase (pH optima 4.5) is shown in Figure 1. Curves for *Humicola insolens* and *Trichoderma longibrachiatum endo*-β-xylanases (pH optima 6.0) are shown in Figures 2 and 3. In each case, the activity of the enzyme preparations employed was determined using wheat arabinoxylan (Lot 20401) (10 mg/mL) as substrate, in either 100 mM sodium acetate buffer (pH 4.5) or sodium phosphate buffer (pH 6.0). The Nelson-Somogyi reducing sugar method, with D-xylose as standard, was used to measure activity.

One unit of enzyme activity is defined as the amount of enzyme required to release one µmole of D-xylose reducing-sugar equivalents from arabinoxylan, at pH 4.5 (or pH 6.0) per minute at 40°C.

milli-Units/assay = 66.6 x Abs.^{2} + 105 x Abs. + 3.9

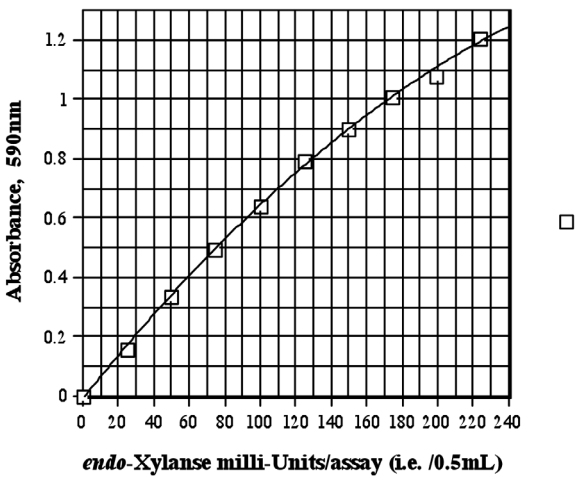

Figure 1. Standard Curve for pure *A. niger* xylanase on Azo-Xylan birchwood (Lot 30601)

NOTE
In the assay formats described here, the substrate concentration has been reduced to 1% w/v, and sodium phosphate buffer is used for assays at pH 6.0. Both of these changes were implemented to improve the cost effectiveness of the assays.

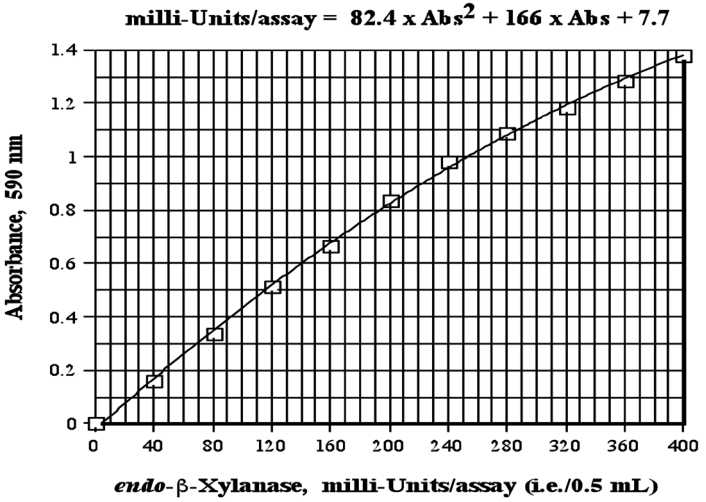

Figure 2. Standard Curve for *Humicola insolens* xylanase preparation on Azo-Xylan birchwood (Lot 30601)

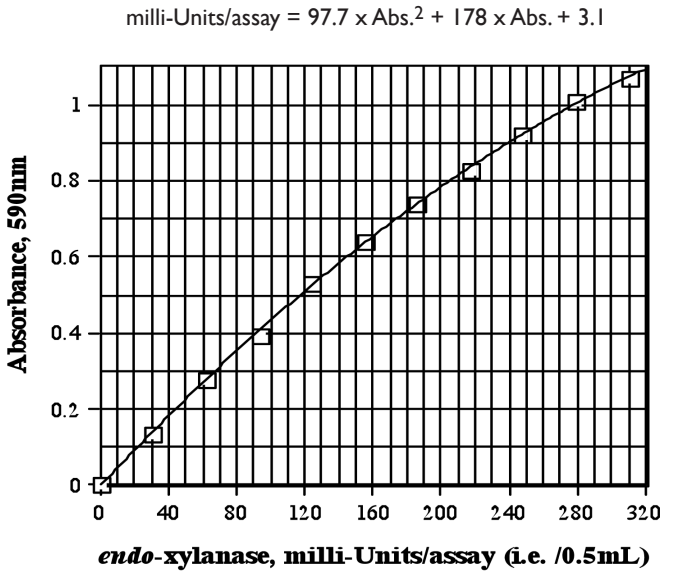

Figure 3. Standard Curve for pure *T. longibrachiatum* xylanase (pI 9.0) on Azo-Xylan birchwood (Lot 30601)

CALCULATION OF ACTIVITY:

Determine *endo*-β-Xylanase activity by reference to the standard curve to convert absorbance values to milli-Units of activity per assay (i.e. per 0.5 mL) on arabinoxylan, and then calculate as follows:

Units/mL or gram of Original Preparation:

$$= \text{milli-Units per assay (i.e. per 0.5 mL)} \times 2 \times 50 \times \frac{1}{1000} \times \text{Dilution}$$

where:

2 = conversion from 0.5 mL to 1.0 mL.

50 = the volume of buffer used to extract the original preparation (i.e. 1.0 g/50 mL or 1.0 mL of enzyme added to 49 mL of buffer).

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

Dilution = further dilution of the original extract.

NOTE: These calculations can be simplified by using the Megazyme ***Mega-Calc***™, downloadable from where the product appears on the Megazyme website (www.megazyme.com).

NOTES:

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