AZO-XYLAN
(Birchwood) (Powder)

PRODUCT INSTRUCTIONS

SKU: 700005043

S-AXBP

02/26

ASSAY OF *endo*-1,4-β-XYLANASE

3 g

Megazyme by NEOGEN

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PRINCIPLE:

This assay procedure is specific for *endo*-1,4-β-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 Remazol Brilliant Blue R™. Before dispensing, the substrate solution should be warmed to room temperature and thoroughly mixed by vigorous shaking. It should be dispensed with a positive displacement dispenser (e.g. Eppendorf® Multipette®).

DISSOLUTION:

Add 1.0 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®).

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 L.

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.

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:

1. Add 0.5 mL of Azo-Xylan birchwood solution (1% w/v) to a 12 x 100 mm glass test tubes and equilibrate at 40°C for 5 min.

2. Pre-equilibrate enzyme solution (~ 5 mL) at 40°C for 5 min.

3. Add 0.5 mL of pre-equilibrated enzyme solutions to tubes containing Azo-Xylan birchwood solution, stir on a vortex mixer for a few seconds and incubate at 40°C for exactly 10 min.

4. Add 2.5 mL laboratory grade ethanol (95%) and stir tube contents vigorously on a vortex mixer for 5 sec to terminate the reaction and to precipitate non-hydrolysed substrate.

5. Store the reaction tubes at room temperature for ~ 10 min and then mix the tubes again. Centrifuge at 1,500 g in a benchtop centrifuge for 10 min.

6. Read the absorbance of all supernatant solutions against the reaction blank at 590 nm and determine the enzyme activity by reference to a standard curve.

7. Prepare a reaction blank by adding 2.5 mL of ethanol (95%) 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.

A standard curve for *A. niger endo*-β-xylanase (pH optima 4.5) is shown in Figure 1. A standard curve for *Trichoderma longibrachiatum endo*-β-xylanases (pH optima 6.0) is shown in Figure 2. In each case, the activity of the enzyme preparations employed was determined using wheat arabinoxylan (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.

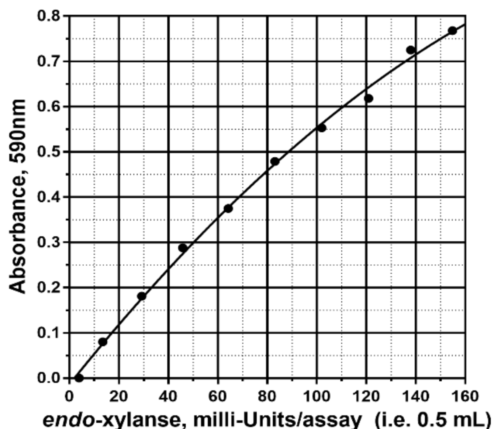

Figure 1. Standard Curve for pure *A. niger* xylanase on Azo-Xylan birchwood (Lot 250917)

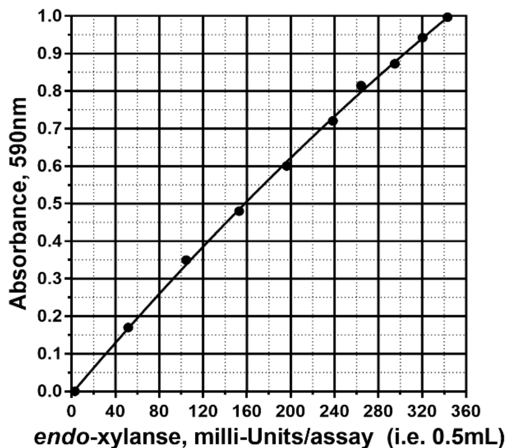

Figure 2. Standard Curve for pure *T. longibrachiatum* xylanase (pI 9.0) on Azo-Xylan birchwood (Lot 250917)

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).

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