

Megazyme

ASSAY OF
endo-1,4- β -Xylanase
using

AZO-XYLAN
(OAT)

S-AXYO 04/06



PRINCIPLE:

This assay procedure is specific for *endo*-1,4- β -D-xylanase activity. On incubation of Azo-Xylan with *endo*-xylanase, the substrate is depolymerised by an *endo*-mechanism to produce low-molecular weight dyed fragments which remain in solution on addition of industrial methylated spirits (IMS, 95 % v/v) or ethanol (95 % v/v) to the reaction mixture. High-molecular weight material is removed by centrifugation, and the colour of the supernatant is measured. *endo*-Xylanase in the assay solution is determined by reference to a Standard Curve.

SUBSTRATE:

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

DISSOLUTION:

Add 2 grams of powder 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 (eg. Eppendorf Multipipette® with a 5.0 mL Combitip).

PRECIPITANT SOLUTION:

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

BUFFER SOLUTION:

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 ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) to 450 mL of distilled water and dissolve. Adjust the pH to 6.0 with 1 M (40 g/L) sodium hydroxide solution. 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 100 mM sodium acetate buffer (pH 4.5). 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 oat) 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 IMS (industrial methylated spirits) or 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.

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.

STANDARD CURVE:

A standard curve for *A. niger* endo- β -xylanase (pH optima 4.5) on Azo-Xylan (oat) Lot 60401, is shown in Figure 1. The activity of the enzyme preparation was determined using wheat arabinoxylan (10 mg/ml) in 100 mM sodium acetate buffer (pH 4.5) as substrate, and using the Nelson-Somogyi reducing sugar method with D-xylose as standard.

One unit of enzyme activity is defined as the amount of enzyme required to release one μ mole of D-xylose reducing-sugar equivalents per minute from wheat arabinoxylan at pH 4.5 and 40°C.

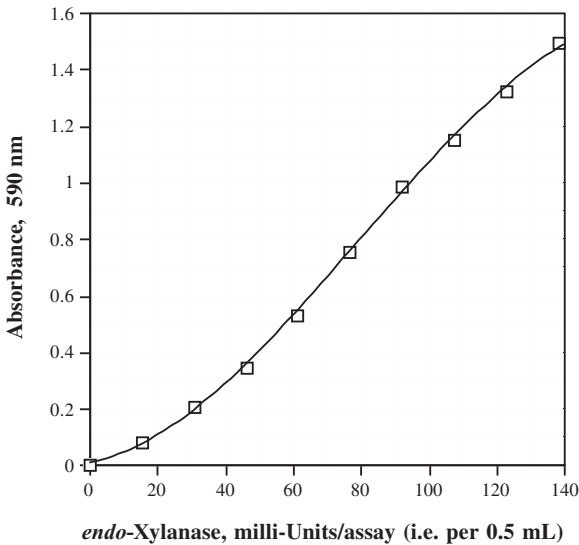


Figure 1. Standard Curve for pure *A. niger* Xylanase on Azo-Xylan (oat) (Lot 60401).

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:

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

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 milliUnits to Units.

Dilution = further dilution of the original extract.



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