

Megazyme

ASSAY OF
endo-XYLANASE
USING
AZO-XYLAN (OAT)

AXO 10/2002



PRINCIPLE:

The 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 about one dye molecule per 30 sugar residues.

DISSOLUTION:

Powdered substrate (2 grams) is added to 80 ml of boiling and vigorously stirring water on a hot-plate stirrer. The heat is turned off and stirring is continued until the solution/slurry is homogeneous (about 20 min). The solution is cooled to room temperature, sodium acetate buffer (5 ml, 2M, pH 4.5) is added, and the pH is adjusted to 4.5, and the volume is adjusted to 100 ml. This solution is stored at 4°C between use. Under these conditions and barring contamination, it is stable for at least 12 months.

The substrate solution should be mixed by shaking before use. The solution is viscous, so it should preferably be dispensed with a positive displacement dispenser (eg. Eppendorf Multipipette® with a 5.0ml Combitip).

BUFFER SOLUTION:

(Sodium Acetate buffer, 100 mM, pH 4.5)

Glacial acetic acid (6.0 g, 1.05 g/ml) is added to 800 ml of distilled water. This solution is adjusted to pH 4.5 by the addition of 5M (20g/100 ml) sodium hydroxide solution. Approximately 50 ml is required. The volume is then adjusted to 1 litre.

PRECIPITANT SOLUTION:

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

ENZYME EXTRACTION AND DILUTION:

Liquid enzyme sample (1.0 ml) is added, using a positive displacement dispenser (these solutions can be very viscous), to **extraction / dilution buffer** (49 ml, pH 4.5) and mixed

thoroughly. This is termed the **Original Extract**. An aliquot of this solution (1.0 ml) is then diluted 10-fold by addition to 9.0 ml of **extraction / dilution buffer**. This process of dilution is repeated until a suitable dilution of the enzyme preparation is achieved. For example, for the industrial enzyme preparations, **Finizym** (from *Aspergillus niger*; Novo Nordisk, Denmark) and **Laminex** (from *Trichoderma* sp.; Genencor International, U.S.A.) a dilution of the original extract of approximately 50-fold is required.

With powder samples, the preparation (1.0 g) is added to **extraction / dilution buffer** (50 ml, pH 4.5) and the slurry is gently mixed over a period of about 15 min or until the sample is completely dispersed or dissolved. This solution (the **Original Extract**) is clarified by centrifugation (1,000 g, 10 min) or filtration through Whatman No. 1 (9 cm) filter circles. This extract is then diluted further with **buffer**, as for the liquid enzyme samples.

ASSAY PROCEDURE:

Pre-equilibrated enzyme solution (0.5 ml) [in 100mM sodium acetate buffer (pH 4.5)] is added to pre-equilibrated substrate solution (0.5 ml), the mixture stirred on a vortex mixer and incubated at 40°C for 10 minutes. The reaction is terminated and high-molecular weight substrate is precipitated by the addition of 2.5 ml of IMS (~95% v/v) with vigorous stirring for 10 seconds on a vortex mixer. The reaction tubes are allowed to equilibrate to room temperature for 5 minutes and are then centrifuged at 3,000rpm (1,500g) for 10 minutes.

The supernatant solution is directly poured into a spectrophotometer cuvette and the absorbance of blank and reaction solutions measured at 590nm. Activity is determined by reference to a Standard Curve.

The blank is prepared by adding IMS to the substrate solution before addition of the enzyme. Usually, only a single blank is required with each set of determinations.

STANDARD CURVE:

A typical standard curve is shown below. This curve is for pure *A.niger* xylanase (Xylanase M4; Megazyme). The activity of the enzyme preparation was determined using wheat arabinoxylan (10 mg/ml) in 100mM sodium acetate buffer (pH 4.5) as substrate, and using the Nelson-Somogyi reducing sugar method with xylose as standard.

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

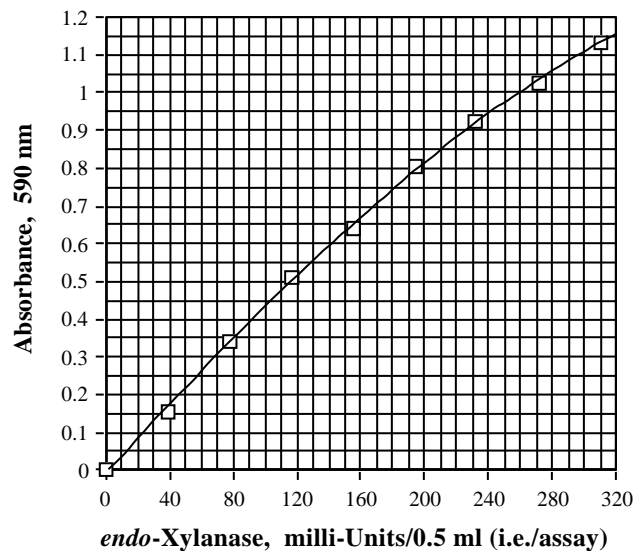


Figure 1. Standard Curve for pure *A.niger* Xylanase on Azo-Xylan (oat) (Substrate Lot 60702)

CALCULATION OF ACTIVITY:

endo-Xylanase activity is determined by reference to the standard curve to convert absorbance to milliUnits of activity per assay (i.e. per 0.5ml) on arabinoxylan, and then calculated as follows:

Units/ml or gram of Original Preparation:

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

where:

2 = conversion from 0.5 ml to 1.0 ml.

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

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

Dilution = further dilution of the original extract.



**Megazyme International Ireland Ltd.,
Bray Business Park, Bray,
Co. Wicklow,
IRELAND**

**Telephone: (353.1) 286 1220
Facsimile: (353.1) 286 1264
Internet: www.megazyme.com
E-Mail: info@megazyme.com**

WITHOUT GUARANTEE

The information contained in this booklet is, to the best of our knowledge, true and accurate, but since the conditions of use are beyond our control, no warranty is given or is implied in respect of any recommendation or suggestions which may be made or that any use will not infringe any patents.