

# Megazyme

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ASSAY OF  
*endo-1,4- $\beta$ -Xylanase*  
using

**AZO-XYLAN  
(BIRCHWOOD)**

AZB 06/2003



**PRINCIPLE:**

This assay procedure is specific for *endo*-1,4- $\beta$ -D-xylanase activity. On incubation of Azo-Xylan (birchwood) 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 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 assay solution is determined by reference to a Standard Curve.

**SUBSTRATE:**

The polysaccharide is purified (to remove starch) and 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 polysaccharide completely dissolves (about 20 min). The volume is adjusted to 100 ml and sodium azide (0.02g) is added and dissolved. This solution is stored at 4°C between use. Under these conditions and barring enzymic 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 Multipette® with a 5.0 ml Combitip).

**PRECIPITANT SOLUTION:**

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

**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.

## 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 5- to 10-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.

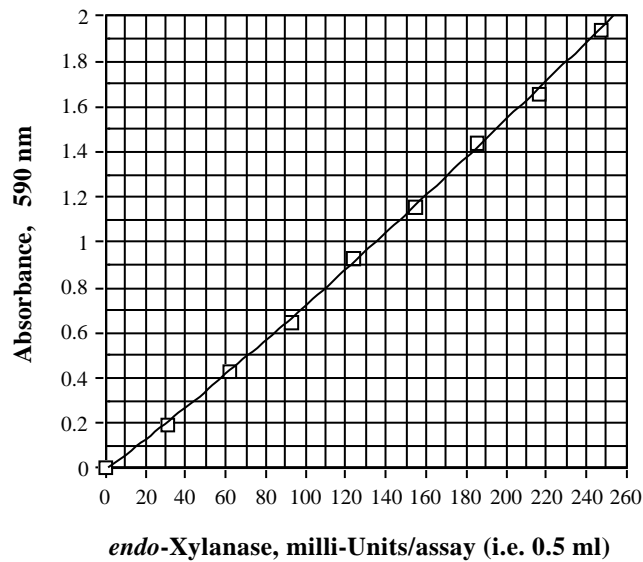
## ASSAY PROCEDURE:

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 is 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 industrial methylated spirits (IMS, 95% v/v) or ethanol (~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 3,000 rpm (1,500 g) for 10 minutes. The supernatant solution is directly poured into a spectrophotometer cuvette and the absorbance of blank and reaction solutions measured at 590 nm. Activity is determined by reference to a Standard Curve.

The reaction blank is prepared by adding IMS (2.5 ml) to the substrate (0.5 ml, 2% w/v) before addition of the enzyme solution (0.5 ml). Usually, only a single blank is required with each set of determinations. Typical blank absorbance values at 590 nm are about 0.07.

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 (medium viscosity Lot 20401) (10mg/ml) in 100 mM 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 arabinoxylan, at pH 4.5 and 40°C.**



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

**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 0.5 ml) on arabinoxylan, and then calculated 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. 1.0g/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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