

# Megazyme

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ASSAY OF  
*endo*-1,4-Beta-Xylanase  
using

**XYLAZYME  
TABLETS**



**SUBSTRATE:**

The substrate employed is Azurine-crosslinked xylan (AZCL-Xylan). The substrate is prepared by dyeing and cross-linking highly purified wheat-flour arabinoxylan to produce a material which hydrates in water but is water insoluble. Hydrolysis by *endo*-(1-4)- $\beta$ -D-xylanase (xylanase) produces water soluble dyed fragments, and the rate of release of these (increase in absorbance at 590 nm) can be related directly to enzyme activity. The substrate is supplied commercially in a ready-to-use tablet form as Xylazyme tablets.

**EXTRACTION BUFFER:**

(Na acetate buffer, 200mM, pH 4.7).

Glacial acetic acid (11.6 g, 1.05 g/ml) is added to 900 ml of distilled water. This solution is adjusted to pH 4.7 by the addition of 2 M (8 g/100 ml) sodium hydroxide solution. Approximately 50 ml is required. The volume is then adjusted to 1 litre.

**DILUTION BUFFER:**

(Na acetate, 25 mM, pH 4.7) containing sodium azide (0.02 %).

Extraction buffer (125 ml) is added to 850 ml of distilled water and sodium azide (0.2 g) is added and dissolved. The pH is adjusted to pH 4.7 by dropwise addition of 2 M hydrochloric acid solution, and the volume is adjusted to 1 litre.

**NOTE:** Do not add the sodium azide until the pH is adjusted. Acidification of sodium azide releases a poisonous gas.

**ENZYME EXTRACTION AND DILUTION:**

Liquid enzyme sample (1.0 ml) is added, using a positive displacement dispenser (these solutions can be very viscous), to Dilution buffer (9 ml, pH 4.7) and mixed thoroughly. An aliquot of this solution (0.5 ml) is then diluted 10-fold by addition to 4.5 ml of 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 Industrias, Denmark) and Laminex (from *Trichoderma* sp.; Genencor International, U.S.A.) a dilution of the original preparation of approximately 10,000-fold is required.

With powder samples, the preparation (1.0 g) is added to Extraction buffer (10 ml, pH 4.7) and the slurry mixed by inversion over a period of 5 min or until the sample is completely dispersed or dissolved. The solution 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 Dilution buffer, as for the liquid enzyme samples.

This procedure can be used to extract xylanase from industrial enzyme preparations, bread improver blends, poultry diets and cereal flours.

### **ASSAY PROCEDURE:**

Aliquots (1.0 ml) of suitably diluted enzyme preparation in sodium acetate buffer (25 mM, pH 4.7) are pre-equilibrated to 40°C for 5 min. Reaction is initiated by the addition of a Xylazyme tablet. The tablet hydrates rapidly. The suspension should not be stirred. After exactly 10 min at 40°C, the reaction is terminated by the addition of Trizma Base solution (10.0 ml, 2 % w/v, Sigma cat. no. T-1503) with vigorous stirring on a vortex mixer. After about 4-5 min standing at room temperature, the slurry is stirred again and then filtered through a Whatman No.1 (9 cm) filter circle.

A substrate/enzyme blank is prepared by adding Trizma Base to the enzyme solution before the addition of the Xylazyme tablet.

**NOTE:** A single blank is required for each set of determinations and this is used to zero the spectrophotometer. The absorbance of the reaction solutions are measured against this blank.

### **STANDARDISATION:**

Standard curves relating the activity of purified *Aspergillus niger* xylanase on wheat arabinoxylan and Xylazyme (Lots 50801 and 70805) are shown in Figure 1. Activity on wheat-flour arabinoxylan was determined at a substrate concentration of 10 mg/ml in 100 mM sodium acetate buffer (pH 4.7) at 40°C using the Nelson/Somogyi reducing sugar procedure.

One Unit of activity is defined as the amount of enzyme required to release one micromole of xylose reducing-sugar-equivalents per minute under the defined assay conditions.

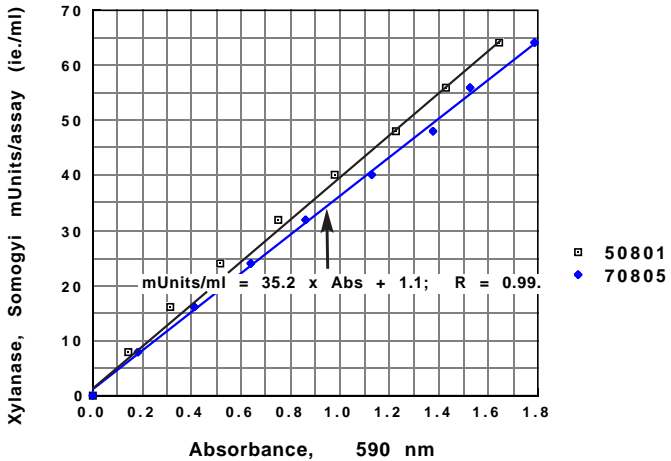


Figure 1. *Aspergillus niger* xylanase standard curve on Xylazyme (70805).

**CALCULATION OF ACTIVITY:**

Xylanase activity is determined by reference to the standard curve or Regression Equation to convert absorbance to milliUnits of activity per assay (i.e. per 1.0 ml), and then calculated as follows:

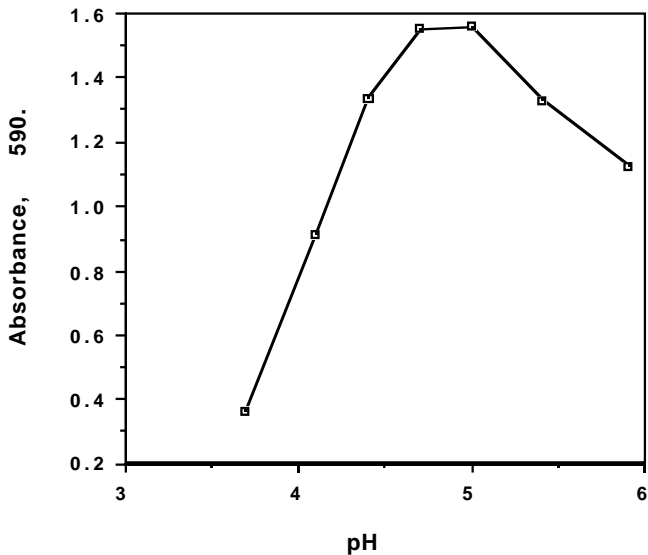
**UNITS/ML OR GRAM OF ORIGINAL PREPARATION:**

$$= \text{milliUnits (per assay ie. per ml)} \times \frac{1}{1000} \times \text{Dilution}$$

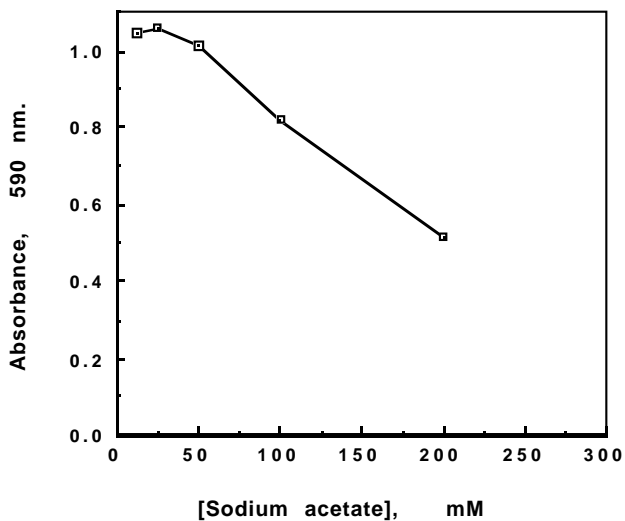
**WHERE:**

1/1000 = conversion from milliUnits to Units.

Dilution = the dilution of the original enzyme preparation.



**Figure 2.** Effect of pH on the activity of *A. niger* xylanase on Xylazyme substrate tablets.



**Figure 3.** Effect of buffer salt concentration on the activity of xylanase On Xylazyme substrate tablets.



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