Megazyme

endo-1,4-ß-XYLANASE

ASSAY PROCEDURES

Xylazyme AF Tablets

T-XAF1000 08/13

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GENERAL INTRODUCTION:

Xylanase (*endo*-1,4-ß-D-xylan xylanohydrolase; EC 3.2.1.8) finds widespread application in chicken feed supplements, in bread improver mixtures and in the enzymic bleaching of wood pulp (Nissen *et al*., 1992[1]). Historically, xylanase activity has been measured with the DNS reducing sugar assay (Bailey, 1988[2]) with purified xylan from oat spelts, larchwood or birchwood as substrate. However, these assays are limited by their lack of sensitivity and linearity, and the fact that they cannot be used to assay activity in materials containing high levels of reducing sugars, e.g. chicken feeds and bread improver mixtures.

In this technical booklet, the use of Xylazyme AF tablets is described. Theses tablets are identical to Xylazyme AX tablets except that they are just 40 mg, compared to Xylazyme AX, which is 60 mg. This smaller size allows more economical use of the substrate in larger scale screening. Repeatability with these tablets is slightly reduced as a result of the lower amount of substrate per test.

SUBSTRATE:

The substrate employed is Azurine-crosslinked wheat arabinoxylan. This substrate is prepared by dyeing and crosslinking highly purified wheat arabinoxylan to produce a material which hydrates in water but is water insoluble. Hydrolysis by *endo*-1,4-ß-D-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 AF tablets.

BUFFERS:

(A) Concentrated Acetate Buffer [Sodium acetate (1 M, pH 4.7)]

Add 60.0 g of glacial acetic acid (1.05 g/mL) to 800 mL of distilled water. Adjust the pH of this solution to pH 4.7 by the addition of 4 M (16 g/100 mL) NaOH solution. Then adjust the volume to one litre. Store at room temperature.

(B) Acetate Extraction/Dilution Buffer [Sodium acetate (25 mM, pH 4.7) containing sodium azide (0.02%)]

Add 25 mL of **concentrated acetate buffer** (A) to 950 mL of distilled water and then add 0.2 g of sodium azide and dissolve. Adjust the pH of the solution to 4.7 by dropwise addition of 2 M HCl acid, and then adjust the volume to one litre.
Store at room temperature.

IMPORTANT NOTE:

Do not add the sodium azide until the pH is adjusted. Acidification of sodium azide releases a poisonous gas. Sodium azide is a poisonous chemical and is added solely as a preservative against microbial infection. This chemical can be deleted from the buffers, but their long-term stability will be reduced.

(C) Concentrated Phosphate Buffer [Sodium Phosphate (0.5 M, pH 6.0)]

Add 156 g of sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$) to 900 mL of distilled water. Adjust the pH to 6.0 with 4 M NaOH. Adjust the volume to 2 litres and add 0.2 g of sodium azide. Store at 4°C.

(D) Phosphate Extraction/Dilution Buffer [Sodium Phosphate (25 mM, pH 6.0)]

Add 50 mL of **concentrated phosphate buffer** (C) to 940 mL of distilled water and add 0.2 g of sodium azide and dissolve. Adjust the pH to 6.0 with 1 M HCl or 1 M NaOH and adjust the volume to one litre. Store at 4°C.

NOTES:

1. Xylazyme AF tablets contain AZCL-wheat arabinoxylan i.e. they are based on wheat arabinoxylan, the native substrate of interest.
2. In the development of Xylazyme AF standard curves, the xylanase activity is first standardised in reducing sugar Units using wheat arabinoxylan and the Somogyi reducing-sugar method.
3. In the assay format described here, 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.
4. The standard curve relating xylanase activity to absorbance at 590 nm is not always linear.
5. Stirring of the test tube on addition of the Xylazyme AF tablet to the enzyme solution gives a slight (about 5%) increase in absorbance values, but it also gives less reproducible results. Consequently, tubes are not stirred after addition of the substrate tablet.
6. AZCL-Wheat arabinoxylan has limited stability in solutions of high pH, consequently Trizma base stopping solution (pH ~ 9) is recommended. When 2% tri-sodium phosphate (pH 11) is used as the stopping reagent, the stopped reaction should be filtered within 5 min of terminating the reaction.

ENZYME EXTRACTION AND DILUTION:

1. Using a positive displacement dispenser, add 1.0 mL of liquid enzyme sample to 99 mL of extraction/dilution buffer B (pH 4.7) or D (pH 6.0) and mix thoroughly. Alternatively, for powder enzyme samples, add 1.0 g to 100 mL of extraction/dilution buffer and stir and extract the slurry over 15 min.
2. Add 0.5 mL of the solution obtained in step 1 to 4.5 mL of extraction/dilution buffer B or D (i.e. dilute 10-fold). Repeat this process of dilution until a concentration suitable for assay is achieved. For example, certain industrial enzyme preparations require a dilution of the **original preparation** of 10,000-fold.

STOPPING REAGENTS:

A. Trizma Base Solution (2% w/v; pH ~ 8.5-9.0)
Dissolve 20 g of trizma base (Sigma Cat. no. T-1503) in 1 Litre of distilled water. Store this solution at room temperature.

B. Tri-Sodium Phosphate [($Na_3PO_4.12H_20$); 2% w/v, pH 11]
Dissolve 20 g of tri-sodium phosphate in 900 mL of distilled water. Adjust the pH to 11.0 with 4 M HCl and the volume to 1 Litre. Store this solution at room temperature.

EQUIPMENT (RECOMMENDED):

1. Glass test tubes (16 x 120 mm, ~16 mL capacity, round bottomed).
2. Micro-pipettors, 1,000 µL (e.g. Gilson Pipetman®).
3. Positive displacement pipettor, e.g. Eppendorf Multipette® with 5.0 mL Combitip® (to dispense 0.50 mL aliquots of enzyme concentrate).
4. Adjustable volume dispenser set at 10.0 mL (for Trizma base or tri-sodium phosphate).
5. Bench centrifuge (required speed 3,000 rpm)(approx. 1,500 *g*).
6. Analytical and top-pan balances.
7. Vortex mixer (e.g. IKA MS2® Minishaker).
8. Thermostated water bath set at 40.0°C (e.g. Julabo PC).
9. Stop clock.
10. Whatman No. 1 (9 cm) filter circles and filter funnels.
11. Spectrophotometer set at 590 nm.

ASSAY PROCEDURE:

1. Add 0.50 mL aliquots of suitably diluted enzyme preparation [in sodium acetate buffer (25 mM, pH 4.7) or sodium phosphate buffer (25 mM, pH 6.0)] to the bottom of glass test tubes (16 x 120 mm) and equilibrate at 40°C for 5 min.
2. Add a Xylazyme AF test tablet to initiate the reaction. The tablet rapidly hydrates. **Do not stir** the suspension.
3. Terminate the reaction exactly 10 min after the addition of the tablet by adding 10.0 mL of Trizma base solution (2% w/v, pH ~ 9.0); or 10.0 mL of tri-sodium phosphate solution (2% w/v, pH 11.0). Stir the tube vigorously on a vortex mixer.
4. Leave the tubes sit at **room temperature** for about 5 min and then stir the contents again. Filter the slurry through a Whatman No. 1 (9 cm) filter circle.
5. Measure the absorbance of the filtrate at 590 nm against a **substrate/enzyme** blank. The **substrate/enzyme** blank is prepared by adding 2% Trizma base solution or tri-sodium phosphate solution to the enzyme solution before the addition of the tablet. The slurry must be left at **room temperature**.

STANDARDISATION:

A Standard curve relating the activity of pure xylanases from *A. niger* on Xylazyme AF tablets (Abs.590 nm) and on wheat arabinoxylan (reducing sugar units) is shown in Figs. 1. Details of the Somogyi reducing sugar assay are given in the Xylazyme AX booklet.

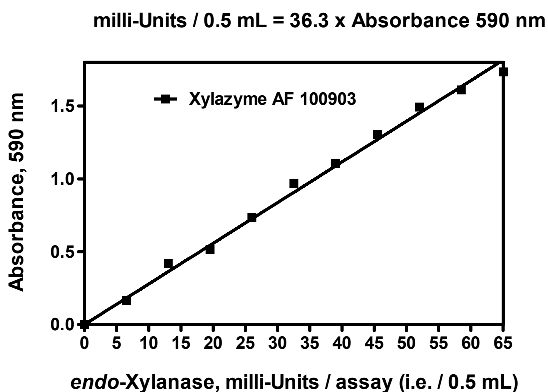

Figure 1. Pure *A. niger* xylanase standard curve on Xylazyme AF tablets (Lot 100903) at pH 4.7. Reaction stopped with Trizma base.

CALCULATION OF ACTIVITY:

Xylanase activity is determined by reference to the standard curve or regression equation to convert absorbance (590 nm) into milli-Somogyi Units of activity per assay, and then calculated as follows:

Units/mL or gram of original preparation:

$$= \text{milli-Units (per assay, i.e. per 0.5 mL)} \times \frac{1}{1000} \times 100 \times 2 \times \text{Dilution}$$

where:

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

100 = initial extraction volume (i.e. 100 mL per g of solid).

2 = conversion from volume assayed (0.5 mL) to 1 mL of extract.

Dilution = further dilution of the initial extraction solution.

NOTE: The regression equation shown in Fig. 1 should be used only over the absorbance range 0.2 - 1.8.

NOTES:

Megazyme International Ireland,
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

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