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ASSAY OF

endo-1,4-β-Xylanase

using

AZO-WHEAT ARABINOXYLAN

S-AWAXP
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PRINCIPLE:

This assay procedure is specific for *endo*-1,4-β-D-xylanase activity. On incubation of Azo-Wheat Arabinoxylan 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 substrate is prepared by dyeing highly purified, and partially depolymerised, wheat arabinoxylan with Remazol Brilliant Blue dye. The substrate is supplied either in a powder or in a soluble "ready-to-use" form. Before dispensing, the substrate should be warmed to room temperature and thoroughly mixed by vigorous shaking. It should be dispensed with a positive displacement dispenser (e.g. Eppendorf Multipette®).

DISSOLUTION (powder substrate):

Add powdered substrate (1 g) to 100 mL of boiling and vigorously stirring water on a hot-plate stirrer. Turn the heat off before adding the substrate. Continue stirring the solution until the powder completely dissolves (about 15 min). Cool the solution to room temperature and adjust the volume to 100 mL. Add sodium azide (0.02 g) as a preservative. Store at 4°C between use. Under these conditions, the substrate will be stable for several years if it is not contaminated with enzyme.

PRECIPITANT SOLUTION:

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

BUFFER SOLUTIONS:

A. 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 by the addition of 5 M (20 g/100 mL) sodium hydroxide solution. Approx. 50 mL is required. Adjust the volume to 1 L.

B. MES Buffer (100 mM, pH 6.0)

Add 21.3 g of MES (Sigma M5287) to 900 mL of distilled water and adjust pH to 6.0 with 5 M (20 g/100 mL) sodium hydroxide solution. Adjust the volume to 1 L.

ENZYME EXTRACTION AND DILUTION:

Using a positive displacement dispenser (these solutions can be very viscous) add liquid enzyme sample (1.0 mL) to **Extraction/Dilution buffer** (49 mL, pH 4.5 or pH 6.0) and mix thoroughly. This is termed the **Original Extract**. Dilute 1.0 mL of this solution 10-fold by addition to 9.0 mL of **Extraction/Dilution buffer A or B**. Repeat this process until a dilution suitable for assay is obtained. 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 100-fold is required.

With powder samples, add 1.0 g of the preparation to 50 mL of **Extraction/Dilution buffer** A or B (pH 4.5 or 6.0) and gently mix the slurry over a period of about 15 min or until the sample is completely dispersed or dissolved. Clarify this solution (the **Original Extract**) by centrifugation (1,500 *g*, 10 min) or filtration through Whatman No. 1 (9 cm) filter circles. Dilute this extract further with **buffer A or B,** to obtain a dilution suitable for assay (as for the liquid enzyme samples).

ASSAY PROCEDURE:

Add 0.5 mL of suitably diluted and pre-equilibrated enzyme solution in 0.1 M sodium acetate buffer (pH 4.5) [or 0.1 M MES buffer (pH 6.0)] to 0.5 mL of pre-equilibrated substrate solution. Stir the mixture and incubate at 40°C for exactly 10 min. Terminate the reaction and precipitate unhydrolysed substrate by the addition of 2.5 mL of laboratory grade ethanol (95%) with vigorous stirring on a vortex mixer. Allow the tubes to equilibrate to room temperature for 10 min, and then mix the tubes again and centrifuge at 1,500 *g* in a benchtop centrifuge for 10 min. Measure the absorbance of the supernatant solution at 590 nm and determine the enzyme activity by reference to a standard curve.

Prepare a reaction blank by adding 2.5 mL of ethanol (95%) 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 ~ 0.05.

One unit of enzyme activity is defined as the amount of enzyme required to release one micromole of xylose reducing-sugar equivalents from wheat arabinoxylan (1% w/v) in one minute at 40°C and pH 4.5 (or pH 6.0).

A typical standard curve for *Aspergillus niger* xylanase on Azo-wheat arabinoxylan (Lot 20601) is shown in Figure 1. To use this Standard Curve, the assay conditions described above must be strictly adhered to.

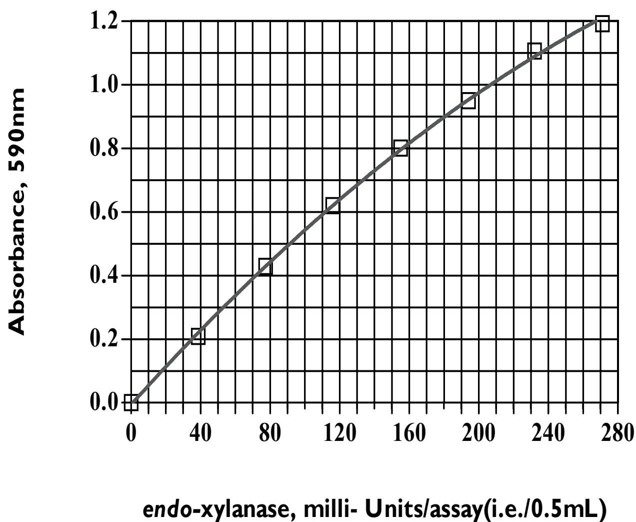

Figure. 1. Standard curve for *Aspergillus niger endo*-xylanase on Azo-wheat arabinoxylan (Lot 20601). Assay conditions are described in the text.

CALCULATION OF ACTIVITY:

Determine ***endo*-Xylanase** activity 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 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.

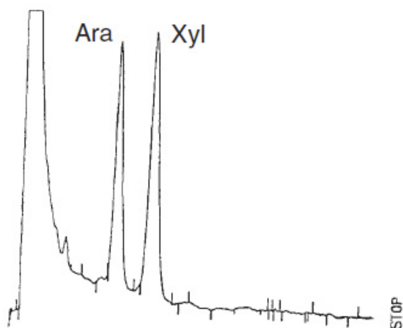

Figure. 2. Glc trace of the alditol acetates of acid hydrolysed wheat-flour arabinoxylan. Ara:Xyl = 41:59.

NOTES:

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