

AZO-WHEAT ARABINOXYLAN (Powder)

PRODUCT INSTRUCTIONS

SKU: 700005042
S-AWAXP

02/26

ASSAY OF *endo*-1,4- β -XYLANASE

3 g



Megazyme
by **NEOGEN**

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 in a powder form. Before dispensing, the substrate solution 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 (1% w/v):

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 (not supplied):

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

PREPARATION OF BUFFERS (not supplied):**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 (**B-MES250; SKU 700004160**) 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 A or B** (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.

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 **Extraction/Dilution buffer A or B**, to obtain a dilution suitable for assay (as for the liquid enzyme samples).

ASSAY PROCEDURE:

1. Add 0.5 mL of Azo-wheat arabinoxylan solution (1% w/v) to a 12 x 100 mm glass test tubes and equilibrate at 40°C for 5 min.
2. Pre-equilibrate enzyme solution (~ 5 mL) at 40°C for 5 min.
3. Add 0.5 mL of pre-equilibrated enzyme solutions to tubes containing Azo-Wheat Arabinoxylan solution, stir on a vortex mixer for a few seconds and incubate at 40°C for exactly 10 min.
4. Add 2.5 mL laboratory grade ethanol (95%) and stir tube contents vigorously on a vortex mixer for 5 sec to terminate the reaction and to precipitate non-hydrolysed substrate.
5. Store the reaction tubes at room temperature for ~ 10 min and then mix the tubes again. Centrifuge at 1,500 *g* in a benchtop centrifuge for 10 min.
6. Read the absorbance of all supernatant solutions against the reaction blank at 590 nm and determine the enzyme activity by reference to a standard curve.
7. 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.

CALCULATION OF ACTIVITY:

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 251143) is shown in Figure 1. To use this Standard Curve, the assay conditions described above must be strictly adhered to.

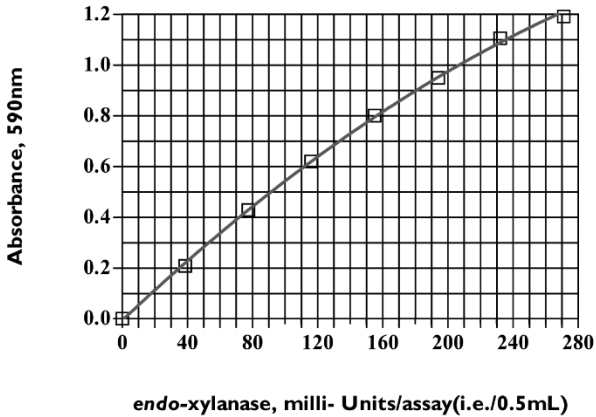


Figure 1. Standard curve for *Aspergillus niger* endo-xylanase on Azo-wheat arabinoxylan (Lot 251143).

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.



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