XYLAZYME TABLETS

PRODUCT INSTRUCTIONS

SKU: 700005112
T-XYZ

09/25

ASSAY OF *endo*-1,4-β-XYLANASE

200 Tablets / 1000 Tablets

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Megazyme by NEOGEN

SUBSTRATE:

Xylazyme tablets are a preparation of azurine-crosslinked arabinoxylan (AZCL-Arabinoxylan), which 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)-β-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, Xylazyme tablets.

REQUIRED REAGENTS (NOT SUPPLIED):

BUFFER CONCENTRATE:

Sodium acetate buffer, 200 mM, pH 4.7

Add 12.1 g of glacial acetic acid (1.05 g/mL) to 900 mL of distilled water. Adjust the pH of this solution to 4.7 by the addition of 2 M (8 g/100 mL) sodium hydroxide solution (approx. 50 mL is required). Adjust the volume to 1 L.

EXTRACTION/DILUTION BUFFER:

Sodium acetate buffer, 25 mM, pH 4.7

Add 125 mL of buffer concentrate to 850 mL of distilled water and adjust the pH 4.7 if necessary by dropwise addition of 2 M hydrochloric acid or 2 M of sodium hydroxide solution. Add 0.5 g of BSA. Adjust the volume to 1 L with distilled water. This is the **Extraction/Dilution buffer**.

Tris buffer 2% w/v, pH 10.0

Add 20 g of Tris Buffer Salt (cat. no. B-TRIS500, SKU 700004162) to 900 mL of distilled water and dissolve. Check the pH and adjust to approx. 10 if necessary. Adjust the volume to 1 L with distilled water. This is **2% w/v Tris buffer solution.**

ENZYME EXTRACTION AND DILUTION:

Liquid samples:

Using a positive displacement dispenser, add 1 mL of liquid enzyme preparation to 99.0 mL of **Extraction/Dilution buffer** and mix thoroughly. Dilute an aliquot of this solution 10-fold by transferring 0.5 mL to 4.5 mL of **Extraction/Dilution buffer**. Mix thoroughly. Repeat this process of 10-fold dilution until a concentration of enzyme suitable for assay is obtained.

Powder samples:
Add 1.0 g of the material to 100 mL of **Extraction/Dilution buffer** and mix on a magnetic stirrer for 10 min, or until the sample is completely dispersed or dissolved. Clarify the solution by centrifugation (1,000 *g*, 10 min) or filtration through Whatman® No. 1 (9 cm) filter circles. Dilute an aliquot of this solution 10-fold by transferring 0.5 mL to 4.5 mL of **Extraction/Dilution buffer.** Mix thoroughly. Repeat this process of 10-fold dilution until a concentration of enzyme suitable for assay is obtained.

ASSAY PROCEDURE:

1. Pre-equilibrate an aliquot (1.0 mL) of suitably diluted enzyme preparation in **Extraction/Dilution buffer** at 40°C for 5 min.
2. Initiate the reaction by adding a Xylazyme tablet. The tablet hydrates rapidly. Do not stir the suspension. Incubate at 40°C for exactly 10 min.
3. Terminate the reaction by adding 10.0 mL of **2% w/v Tris buffer solution**, with vigorous stirring on a vortex mixer.
4. Leave the tubes at room temperature for about 5 min, and then stir them again.
5. Filter the slurry through a Whatman No. 1 (9 cm) filter circle.
6. Measure the absorbance of the filtrate at 590 nm against a substrate blank.

If the absorbance is above 2.0, dilute an aliquot of the enzyme extract with an equal volume of **Extraction/Dilution buffer** and repeat the assay.

Prepare a **substrate blank** by adding 10 mL of **2% w/v Tris buffer solution,** to the enzyme solution before adding the Xylazyme tablet. After adding the tablet, stir the tube vigorously and allow the tube to stand at room temperature for approx. 10 min. Then filter the slurry through Whatman No. 1 filter paper.

NOTE: A single substrate 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:

A standard curve relating the activity of purified *Aspergillus niger* xylanase on wheat arabinoxylan and Xylazyme Tablets is shown in Figure 1. Xylanase activity was standardised using wheat arabinoxylan (10 mg/mL) in 100 mM sodium acetate buffer (pH 4.7) as substrate, performing incubations at 40°C and measuring increase in reducing sugar level using the Nelson/Somogyi reducing sugar procedure.

One Unit of activity is defined as the amount of enzyme required to release one micromole of D-xylose reducing-sugar-equivalents from wheat arabinoxylan (10 mg/mL) in 100 mM sodium acetate buffer (pH 4.7) per minute at 40°C.

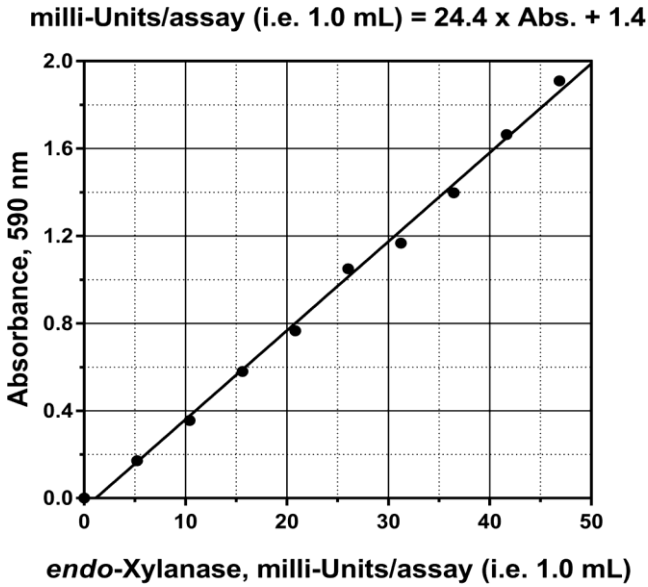

Figure 1. Standard curve for *Aspergillus niger* xylanase on Xylazyme tablets (T-XYZ-200T lot 190901 and T-XYZ-1000T lot 250735)

CALCULATION OF ACTIVITY:

Xylanase activity is determined by reference to the standard curve or regression equation to convert absorbance to milli-Units of activity per assay (i.e. per 1.0 mL), and then calculated as follows:

Units/mL or per gram of Original Preparation:

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

where:

1/1000 = conversion from milli-Units to Units.
Dilution = the dilution of the original enzyme preparation.

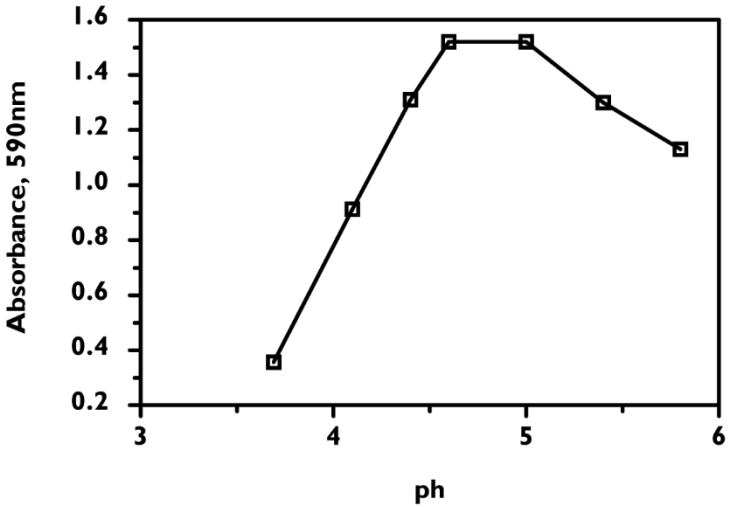

Figure 2. Effect of pH on the activity of *A. niger* xylanase on Xylazyme tablets.

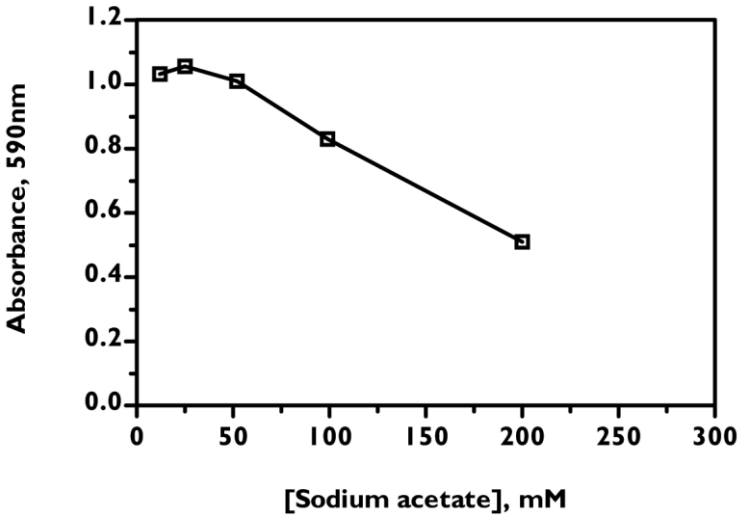

Figure 3. Effect of buffer salt concentration on the activity of *A. niger* xylanase on Xylazyme substrate tablets.

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