

# Megazyme

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MEASUREMENT OF  
**XYLANASE**  
IN ANIMAL FEEDS

using

**AZO-WAX  
SOLUTION**

K-AZOWAX 09/04

(200 Assays per Kit)



## INTRODUCTION:

Arabinoxylan is the major endosperm cell-wall polysaccharide of wheat and rye and is found in significant proportions in most cereal solutions and slurries of high viscosity and, in animal nutrition, it reduces the rate of nutrient absorption from the gut.

*endo*- $\beta$ -D-Xylanase (xylanase) is added to feeds to catalyse depolymerisation of this polysaccharide. It can be demonstrated that *endo*-cleavage by xylanase of just one bond per thousand in the arabinoxylan backbone can significantly remove viscosity properties.

Of the carbohydrase enzymes used as feed supplements, one of the most difficult to measure has been xylanase. These problems are attributed to several factors, including the low levels of enzyme added to the feed, inactivation of enzyme during pelleting, binding of the enzyme to feed components and the presence of specific xylanase inhibitors.

The only biochemical methods which are sufficiently sensitive, specific and robust to measure xylanase in feeds are viscometric assays and those employing dyed xylan or arabinoxylan polysaccharides.

Viscometric assays are tedious, whereas assays employing dyed xylan substrates are rapid, reproducible and simple to perform. We recommend the use of either Xylazyme AX tablets or Azo-Wheat Arabinoxylan (Azo-WAX). Xylazyme AX based assays are about 5-fold more sensitive than assays employing Azo-WAX. However, this latter substrate does not have sufficient sensitivity in most applications, and results are slightly more reproducible than with Xylazyme AX.

It is generally accepted that xylanase enzymes which are best suited to feed applications have optimal activity at pH 6.0. Consequently, these enzymes are generally assayed at this pH in 100 mM sodium phosphate buffer. In recovery experiments, however, we found that sodium phosphate buffer extracts only a small proportion (< 20%) of the amount of enzyme added to the feed. Thus a wide range of alternative extractants and extraction conditions have been evaluated. For feeds containing *Trichoderma* sp. xylanases, the best and most consistent results have been obtained using 100 mM acetic acid or 100 mM sodium acetate buffer (pH 4.7) at room temperature. Optimal extraction of *Humicola* sp. xylanases was achieved with a buffer containing 100 mM MES buffer (pH 6.0) and 1% w/v sodium dodecyl sulphate (SDS).

## KITS:

Kits containing the required reagents to measure xylanase in animal feeds are available from Megazyme. These kits contain:

1. Azo-wheat arabinoxylan (100 mL, 1% w/v).
2. *Trichoderma sp.* control xylanase [3700 milli-Units (mU)/mL; pH 6.0 and 40°C] in 50% glycerol.

## EXTRACTION BUFFERS (not enclosed):

### (A) Sodium acetate buffer (100 mM, pH 4.7)

Add 6.0 g of glacial acetic acid (1.05 g/mL) to 800 mL of distilled water. Adjust the pH to 4.7 with 2 M (8 g/100 mL) sodium hydroxide solution, and the volume to 1 litre. Add 0.2 g of sodium azide and dissolve. Stable at room temperature for > 12 months.

### (B) MES buffer (100 mM) plus SDS (1% w/v)

Add 19.5 g of MES free acid (Sigma M-8250) to 800 mL of distilled water and dissolve. Adjust the pH to 6.0 with 1 M sodium hydroxide. Add 10 g of SDS (sodium lauryl sulphate; Sigma L-4509) and dissolve. Adjust the volume to 1 litre and add 0.2 g of sodium azide and dissolve. Stable at room temperature for > 12 months.

## EQUIPMENT (Recommended):

1. Glass test tubes (round bottomed; 16 x 100mm and 16 x 120mm).
2. Micro-pipettor, e.g. Gilson Pipetman® (200 µL and 500 µL).
3. Positive displacement pipettor e.g. Eppendorf Multipette®
  - with 5.0 mL Combitip® (to dispense 0.5 mL aliquots of Azo-WAX solution).
  - with 25 mL Combitip® (to dispense 2.5 mL aliquots of IMS or ethanol).
4. Analytical balance.
5. Spectrophotometer set at 590 nm.
6. Vortex mixer (e.g. IKA® Yellowline Test Tube Shaker TTS2).
7. Stop clock.
8. Whatman No.1 (9 cm) filter papers.

## EXTRACTION AND ASSAY OF XYLANASE IN FEED SAMPLES:

### *Trichoderma* sp. Xylanases:

#### EXTRACTION:

1. Mill feed samples (approx. 50 grams) to pass a 0.5 mm screen and mix thoroughly.
2. Weigh 0.5 g  $\pm$  (0.01 g) of each sample in duplicate into glass test-tubes (16 x 120 mm).
3. Add 5 mL of 100 mM sodium acetate buffer (pH 4.7) to each sample and stir on a vortex mixer. Add 0.2 mL of water to one tube and 0.2 mL of xylanase ( $\sim$  740 mU) to the other. Immediately stir the slurries on a vortex mixer.
4. Incubate the slurries at room temperature and stir occasionally over the following 20 min.
5. Centrifuge the tubes at 1,500 g for 10 min in a bench centrifuge and use the supernatant directly in assays. Start assays within 30 min of obtaining these extracts to minimise loss of enzyme activity.

#### ASSAY:

1. Accurately transfer 0.5 mL aliquots of supernatant solutions (in duplicate) to glass test-tubes (16 x 100 mm) **at room temperature**.
2. Add 0.5 ml of Azo-WAX (1% w/v) to each tube and stir the tube vigorously. Immediately place the tubes in a water bath and incubate at  $50^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$  for exactly 30 min.
3. Add 2.5 mL of IMS or ethanol and stir the tube vigorously on a vortex mixer. Store the tube at **room temperature** for 5 min.

**This treatment terminates the reaction and precipitates non-depolymerised dyed substrate.**

4. Centrifuge the tubes at 1,500 g for 10 min.
5. Measure the absorbance of the supernatant solutions at 590 nm against a reaction blank.

Prepare a Reaction Blank by adding 2.5 mL of IMS or ethanol to a mixture of 0.5 mL of Azo-WAX and 0.5 mL of 100 mM sodium acetate buffer (pH 4.7). Stir the slurry and store at room temperature for 5 min before centrifugation (1,500 g, 10 min). A single reaction blank is required for each feed sample.

### CALCULATION OF ACTIVITY:

The level of xylanase in the flour sample is calculated as follows:

$$\text{Activity in feed sample (0.5 g)} = \text{Added activity} \times \frac{\text{SA}}{\text{TA} - \text{SA}}$$

#### where:

- Added activity = the amount of xylanase added to the feed slurry at the time of assay e.g.: 740 mU in the control xylanase solution (0.2 mL).
- SA = the reaction absorbance obtained for extracts of the feed to which no control xylanase was added.
- TA = the total absorbance, i.e. the absorbance of extracts of the sample to which the control xylanase was added.

### EXAMPLE CALCULATION:

Sample	Abs/30 min. incubation
1. Feed A	0.000
2. Feed A containing <i>Trichoderma</i> sp. xylanase ( <b>SA</b> )	0.340
3. <b>SA</b> + 740 mU xylanase (in the assay) ( <b>TA</b> )	0.798

$$\text{Activity in 0.5 g of feed A} = \text{Added activity} \times \frac{\text{SA}}{\text{TA} - \text{SA}}$$

#### where:

- SA = absorbance of **extract of sample A** [assayed by the standard format (e.g. 0.340)]
- TA = **total absorbance**, i.e. the absorbance of extracts of **sample A** to which the additional xylanase (0.2 mL; 740 mU) was added (e.g. Abs = 0.798).







**Megazyme International Ireland,  
Bray Business Park, Bray,  
Co. Wicklow,  
IRELAND.**

**Telephone: (353.1) 286 1220**

**Facsimile: (353.1) 286 1264**

**Internet: [www.megazyme.com](http://www.megazyme.com)**

**E-Mail: [info@megazyme.com](mailto:info@megazyme.com)**

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