CREATIVE BIOMART®
Assay Kit

Xylanase Assay Kit (Xylazyme AX)

Product Information

Common Name

Xylanase

Cat.No.

Kit-0849

Product Overview

For the measurement and analysis of endo-1,4-β-D-xylanase in enzyme preparations, bread improver mixtures and animal feeds. Contains Xylazyme AX Tablets and xylanase enzyme controls (A. niger and Trichoderma longibrachiatum).

Size

200 assays

Kit Components

1. Xylazyme AX test tablets (200 tablets).
2. A. niger control xylanase (~ 295 mU/mL at 40°C and pH 4.7) in 50 % (v/v) glycerol (activity stated on vial).
3. T. longibrachiatum control xylanase (~ 386 mU/mL at 40°C and pH 6.0) in 50% (v/v) glycerol (activity stated on vial).

Materials Required but Not Supplied

1. Glass test tubes (round bottomed; 16 x 100 mm and 16 x 120 mm).
2. Micro-pipettors e.g.: Gilson Pipetman 200 μL and 500 μL.
3. Positive displacement pipettor e.g.: Eppendorf Multipette®- with 5.0 mL Combitip® [to dispense 0.2 mL aliquots of xylanase
control in 50% (v/v) glycerol].
4. Adjustable volume dispenser set at 5.0 mL (to dispense Trizma base solution).
5. Top-pan balance correct to 0.01 g.
6. Spectrophotometer set at 590 nm.
7. Vortex mixer (e.g. IKA® Yellowline Test Tube Shaker TTS2).

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8. Whatman No. 1 (9 cm) filter circles and filter funnels.

Scientific Background

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-β-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 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).

Detection method Based on use of Xylazyme AX tablets (590 nm)

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Features & Benefits

Very cost effective
All reagents stable for > 2 years during use

Preparation

EXTRACTION BUFFERS: (not enclosed):
(A) Acetic acid (0.1 M)
Add 5.8 mL of glacial acetic acid (1.05 g/mL) to 900 ml of distilled water and adjust the volume to 1 litre. 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 to 800 mL of distilled water and dissolve. Adjust the pH to 6.0 with 1 M sodium hydroxide. Add 10 g of sodium lauryl sulphate (SDS) 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.

Assay Protocol

EXTRACTION:
1. Mill feed samples (approx. 50 g) to pass a 0.5 mm screen and mix thoroughly.
2. Weigh 0.5 g (± 0.01 g) of each sample in quadruplicate into glass test-tubes (16 x 120 mm).
3. Add 5 mL of 0.1 M acetic acid to each sample and stir on a vortex mixer. Add 0.2 mL of distilled to two of these tubes with stirring. To the other two tubes add 0.2 mL of control xylanase solution (approx. 60-80 mU/0.2 mL; see vial label) with vigorous and immediate stirring 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 the assays. Assays should be initiated within 30 min of obtaining these extracts to minimise loss of enzyme activity in the extracts.
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 a Xylazyme AX tablet (without stirring) to each tube and immediately place the tubes in a water bath set at 50 ± 0.1°C and incubate for exactly 30 minutes.

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3. After exactly 30 minutes, add 5 mL of Trizma Base solution (pH ~ 9), stir vigorously on a vortex mixer and store at room temperature for 5 minutes.
NOTE:
1. This treatment terminates the reaction.
2. The tubes must be stored at room temperature and not at 50°C, as the substrate is not stable under alkaline conditions at elevated temperatures (i.e.: absorbance values will increase due to substrate breakdown).
4. Stir the tubes on a vortex mixer and filter the slurry through a Whatman No. 1 (9 cm) filter paper.
5. Measure the absorbance of the filtrates at 590 nm against a Reaction Blank.
Prepare the Reaction Blank by adding Trizma Base solution (5 mL) to the feed extract (0.5 mL), followed by a Xylazyme AX tablet. Stir the slurry and store at room temperature for 5 min before filtration through Whatman No. 1 filter paper.
A single Reaction Blank is required for each feed sample.

Assay time

~ 45 min

Analysis

The level of xylanase in the flour sample is calculated as follows:
Activity in feed sample (0.5 g) = Added activity SA/ (TA - SA)
where:
Added activity = the amount of xylanase added to the feed sample slurry at the time of assay e.g.: 70 mU in the control xylanase solution (0.2 mL).
SA = the reaction absorbance obtained for extracts of the feed sample to which no control xylanase was added.
TA = the total absorbance i.e. the absorbance of extracts of the feed sample to which the control xylanase was added.

Sensitivity

0.02 U/mL of assay solution

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