ASSAY OF ENDO-XYLANASE ACTIVITY IN FEED

Principle

The substrate is dyed and cross linked wheat arabinoxylan in tablet form. Hydrolysis by endo-1,4-β-xylanase produces water soluble dyed fragments. The rate of release of these fragments (increase in absorbance at 590 nm) can be related directly to enzyme activity.

Field of application

The method is suitable for measurement of endo-xylanase activity in feed samples without a strong binding ability. If binding at high degree occurs, a spiking method must be applied. The endogenous xylanase activity in feed, if present, increases the result.

Unit of activity

One xylanase unit (BXU) is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one nmol xylose from birch xylan in one second under the assay conditions (1 BXU = 1 nkat). The xylanase activity obtained by the tablet method is declared in F AXU units ("F" refers to feed)

Assay conditions

Substrate	azurin-cross linked wheat arabinoxylan
pH	5.0
Temperature	50 °C ± 0.5 °C
Incubation time	30 min

Equipment

Water bath
Centrifuge
Test tube mixer (vortex)
Spectrophotometer

Reagents

All solutions are prepared in deionized water, Milli-Q or equivalent.

1. Acetate buffer (0.05 M, pH 5.0)
Add 2.85 ml of glacial acetic acid to about 900 ml of water. Adjust to pH 5.0 with NaOH and make the volume to 1000 ml.

2. Xylazyme AX tablets (60 mg tablets, T-XAX) from Megazyme. There might be differences between different tablet lots. All samples in one series should be assayed with tablets of the same lot.

3. Trizma Base 1 %(w/v)
Dilute 10.0 g of Trizma Base (Sigma T-1503) in water and make up to 1000 ml.

Sample

Weigh accurately about 2.5 g of well ground sample (<0.5 mm) in a baker of 50 ml. Add 20 ml of buffer. Keep under magnetic stirring for 30 min at room temperature. Centrifuge about 10 ml of the suspension for 10 min at 4000 rpm. Use the supernatant for the assay (dilute further in buffer if necessary). The absorbance should be 0.2 - 1.5. Normally three replicate analysis shall be done per sample.

Assay

Pipette 1.0 ml of sample to a test tube. Equilibrate for 5 min in a water bath at 50 °C. Reaction is initiated by addition of a Xylazyme AX tablet. The suspension should not be stirred. After exactly 30 min stop the reaction by adding 5.0 ml of Trizma Base with vigorous stirring on a vortex mixer. The tube is left at room temperature for about 5 min; the slurry is stirred again and filtered through Whatman 1 filter paper. The absorbance of the filtrate is measured against enzyme blank at 590 nm. The enzyme blank is prepared by adding Trizma Base to the enzyme solution before the addition of Xylazyme AX tablet. The enzyme blank shall not be incubated at 50 °C.

Calculation

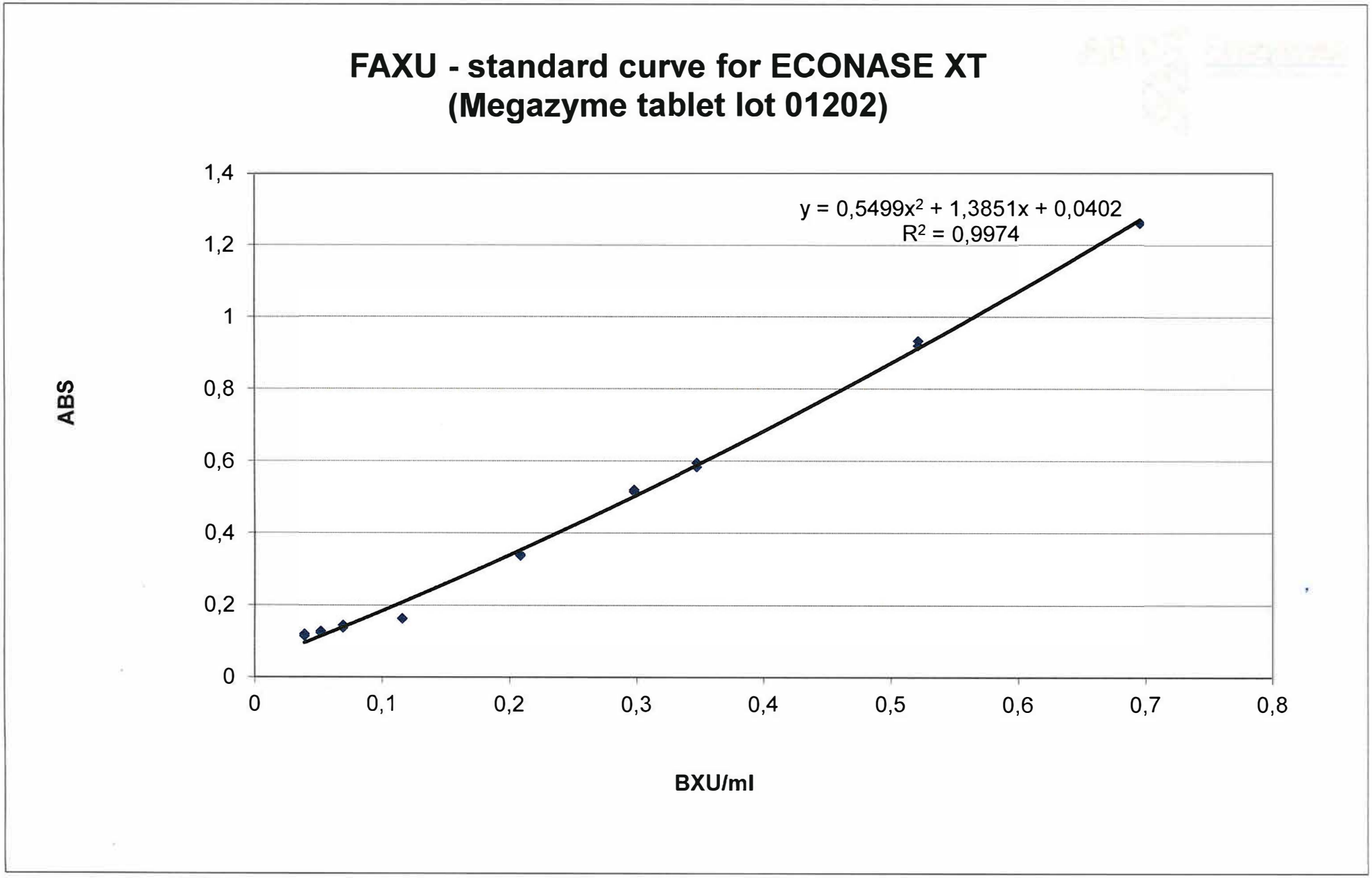
The xylanase activity corresponding to the absorbance is read from the standard curve. The standard curve is obtained by assaying a series of dilutions containing known xylanase activities. The sample used for the standardisation must be the very same as in the feed samples to be analysed.

The xylanase activity FAXU/kg of feed in the sample:

$$\frac{BXU/ml \cdot 20 \cdot k \cdot 1\,000}{m}$$

BXU/ml = the activity from the standard line
20 = the volume of buffer used for extraction (ml)
m = the amount of sample (g)
k = the dilution factor
1 000 = conversion factor from g to kg

Appendix	Example of a standard curve
Reference	Megazyme International Ireland Ltd. Data booklet – Xylazyme AX (Xyl 7/01)


FAXU - standard curve for ECONASE XT
(Megazyme tablet lot 01202)
y = 0,5499x² + 1,3851x + 0,0402
R² = 0,9974
ABS
BXU/ml
1,4
1,2
1
0,8
0,6
0,4
0,2
0
0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
0,8