Megazyme

ASSAY OF

***endo*-1,5-α-L-ARABINANASE**

using

ARABINAZYME TABLETS

T-ARZ200 06/03

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SUBSTRATE:

The substrate employed is Azurine-crosslinked-debranched arabinan (AZCL-Arabinan). Highly purified arabinan from sugar-beet pulp is treated with α-L-arabinofuranosidase to remove 1,3- and 1,2-α–linked arabinofuranosyl residues, leaving linear 1,5-α-L-arabinan. This polysaccharide still contains a small percentage of galacturonic acid, galactose and rhamnose (6, 4 and 2 %, respectively), but is resistant to attack by polygalacturonanase and *endo*-1,4-ß-D-galactanase. The polysaccharide is then dyed and crosslinked. Treatment of this substrate with a large excess of α-L-arabinofuranosidase results in a limited release of arabinose but no release of dye labelled fragments.

AZCL-Arabinan is a highly sensitive and very specific substrate for the assay of *endo*-arabinanase and can be used to measure this activity in the presence of large excesses of other pectin degrading enzymes and in the presence of sugars and other stabilisers present in industrial powder and liquid pectinase enzyme preparations. This substrate is supplied commercially in a ready-to-use form as **Arabinazyme tablets.**

EXTRACTION/DILUTION BUFFER:
[(Sodium Acetate buffer, 50 mM, pH 4.0) containing sodium azide (0.02%)]

Glacial acetic acid (3.05 g, 1.05 g/mL) is added to 900 mL of distilled water. This solution is adjusted to pH 4.0 by the addition of 1 M (4 g/100 mL) sodium hydroxide solution. Approximately 12 mL is required.

Sodium azide (0.2 g) is added, the pH is readjusted to pH 4.0 by dropwise addition of 1 M hydrochloric acid, and the volume adjusted to 1 litre. Store at 4°C.

NOTE: Sodium azide is a toxic chemical and should be treated accordingly.

ENZYME DILUTION:

For most commercial pectinase enzyme preparations, a dilution of 500-fold is required. Liquid enzyme preparation (1.0 mL) is added (using a positive displacement dispenser) to **Extraction/Dilution** buffer (9.0 mL). An aliquot of this solution (0.2 mL) is then further diluted by addition to 9.8 mL of sodium acetate buffer (50 mM, pH 4.0).

With powder samples, the preparation (1.0 g) is added to **Extraction/ Dilution** buffer (20 mL) and mixed until either completely dissolved or dispersed. This solution is clarified by centrifugation (1,000 g, 10 min) or filtration through Whatman No.1 filter circles, and then further diluted as for the liquid samples.

If reaction values greater than 1.5 **Absorbance Units** are obtained, the enzyme solution should be diluted a further 5-fold in **Extraction/ Dilution** buffer and the assay repeated.

ASSAY PROCEDURE:

1. Aliquots (0.5 mL) of suitably diluted enzyme in **Extraction/ Dilution** buffer are pre-equilibrated to 40°C for 5 min.
2. Reaction is initiated by the addition of an Arabinazyme tablet. The tablet hydrates rapidly. The suspension should **not** be stirred.
3. After exactly 10 min at 40°C, the reaction is terminated by the addition of Trizma Base solution (10 mL, 2 % w/v, Sigma cat. no. T-1503) with vigorous stirring on a vortex mixer.
4. After about 5 min standing at room temperature, the slurry is stirred again and filtered through a Whatman No.1 (9 cm) filter circle.
5. The absorbance of the reaction solutions are then measured at 590 nm against the reaction blank.

A **substrate/enzyme (reaction) blank** is prepared by adding Trizma Base (10 mL) to the enzyme solution (0.5 mL) before the addition of the Arabinazyme tablet.

A **single blank** is required for each set of determinations and this is used to zero the spectrophotometer.

STANDARDISATION:

A standard curve relating the activity of purified ***endo*-arabinanase** on linear 1,5-α–L-arabinan [carboxymethyl (CM) form] and Arabinazyme (Lot 00401) is shown in Figure 1. The curves obtained for *endo*-arabinanase in the crude commercial enzyme preparations tested, were the same. Activity on CM-linear 1,5-α–L-arabinan was determined at a substrate concentration of 2 mg/mL in 100 mM sodium acetate buffer (pH 4.0) at 40°C using the Nelson/Somogyi reducing sugar procedure. The effects of pH and salt concentrations on measured activity are shown in Figures 2 and 3.

CALCULATION OF ACTIVITY:

1. ***endo*-Arabinanase activity in the sample** being assayed is determined by reference to the standard curve to convert absorbance values to milliUnits per assay (i.e. per 0.5 mL).

Alternatively, for absorbance values in the range of 0.1 to 1.5, these values can be calculated by reference to the equation:

$$Y = MX + C.$$

where:

Y = *endo*-arabinanase activity (in milliUnits/assay).

M = slope of the calibration graph.

X = absorbance of the reaction at 590 nm (minus the reaction blank, or read against the reaction blank).

C = intercept on the Y-axis.

Values for M and C vary slightly between batches of Arabinazyme tablets. M and C values for the particular batch of tablets are provided with the tablets.

2. ***endo*-Arabinanase activity per mL or gram of original preparation:**

$$= Y \times \frac{1}{1000} \times 2 \times \text{Dilution}$$

where:

$\frac{1}{1000}$ = conversion from milliUnits to units.

2 = conversion from 0.5 mL to 1.0 mL.

Dilution = the dilution of the original enzyme preparation (i.e. 200-fold for the Megazyme Control Solution).

One **Unit of activity** is defined as the amount of enzyme required to release one micromole of arabinose reducing-sugar equivalents from CM-linear arabinan per minute under the defined assay conditions. The rate of hydrolysis of CM-linear arabinan by *endo*-arabinanase is the same as that obtained for linear arabinan recovered from pear juice concentrate.

A stabilised ***endo*-Arabinanase Control Solution** can be obtained from Megazyme.

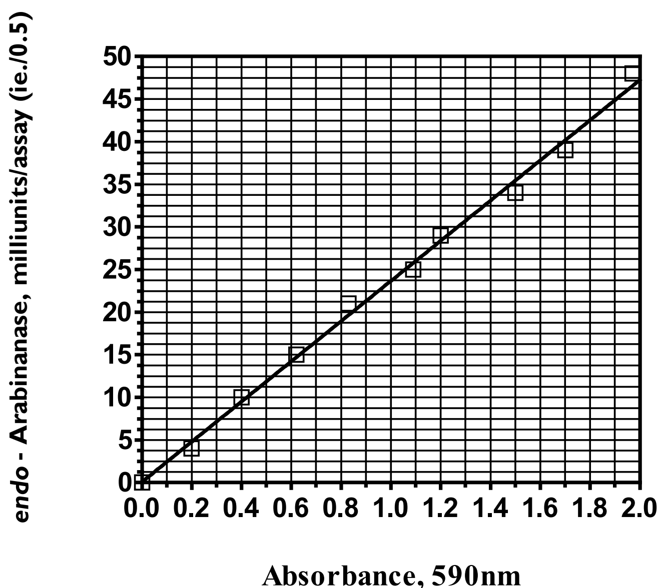

Figure 1. *endo*-Arabinanase standard curve on Arabinazyme tablets (Lot 00401).

endo-Arabinanase (0.5 mL, 0-40 milliUnits) in 50 mM sodium acetate buffer (pH 4.0) containing sodium azide (0.02%) in a glass test-tube (16 x 120 mm) was pre-equilibrated at 40°C for 5 min. The reaction was initiated by the addition of an Arabinazyme tablet without stirring. Reaction was terminated after 10 min by the addition of Trizma Base (10 mL, 2% w/v) with vigorous stirring. After about 5 min, the tubes were stirred again, and the solutions filtered through Whatman No. 1 (9 cm) filter circles, and the absorbance of the filtrate read against an enzyme/substrate blank solution at 590 nm.

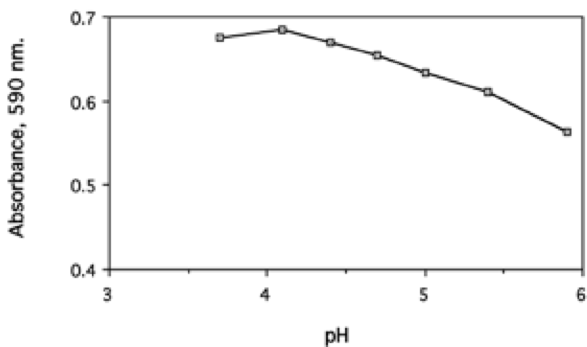

Figure 2. Effect of pH on the activity of *endo*-arabinanase on Arabinazyme tablets.

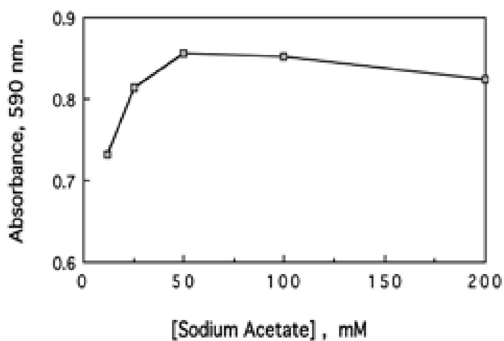

Figure 3. Effect of buffer salt concentration on the activity of *endo*-arabinanase on Arabinazyme tablets

NOTES:

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

Telephone: (353.1) 286 1220
Facsimile: (353.1) 286 1264
Internet: www.megazyme.com
E-Mail: info@megazyme.com

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