ARABINAZYME TABLETS

PRODUCT INSTRUCTIONS

SKU: 700005096
T-ARZ-200T

06/25

ASSAY OF *endo*-1,5-α-ARABINASE

200 Tablets

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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-β-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.**

SAFETY:

The general safety measures that apply to all chemical substances should be adhered to.

Safety data sheets are available for all products at www.megazyme.com.

EXTRACTION/DILUTION BUFFER:
Sodium Acetate buffer (50 mM, pH 4.0).

Add 2.9 mL of glacial acetic acid to 900 mL of distilled water. Adjust to pH 4.0 by the addition of 1 M sodium hydroxide solution. Adjust the volume to 1 L.

STOPPING REAGENT:
Tris buffer salt solution (2% w/v).

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. 8.5 if necessary. Adjust the volume to 1 L with distilled water.

ENZYME DILUTION:

For liquid preparations, use a positive displacement dispenser to transfer 1.0 mL of liquid enzyme preparation to 49.0 mL of Extraction/Dilution buffer and thoroughly mix the solution. Dilute this original extract 10-fold (1 mL to 9 mL of Extraction/Dilution buffer) and further, until a concentration suitable for assay is obtained.

With powder samples, add 1.0 g of powdered enzyme preparation to 50 mL of Extraction/ Dilution buffer and stir on a magnetic stirrer for approx. 15 min at room temperature (until the powder is completely dissolved or dispersed). Filter the preparation through a Whatman™ No. 1 filter paper or centrifuge an aliquot in a microfuge for 3 min at 15,000 g or in a bench centrifuge at ~ 4,000 g for 10 min, if necessary. Dilute this original extract 10-fold (1 mL to 9 mL of Extraction/ Dilution buffer) and further, until a concentration suitable for assay is obtained.

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

ASSAY PROCEDURE:

1. Add 0.50 mL aliquots of suitably diluted enzyme preparation to the bottom of glass test tubes (16 x 120 mm) and equilibrate at 40°C for 5 min.
2. Add an Arabinazyme tablet to initiate the reaction. The tablet hydrates rapidly. The suspension should **not** be stirred.
3. Terminate the reaction **exactly 10 min** after the addition of the tablet by adding 10.0 mL of Tris buffer salt solution (2% w/v, pH ~ 8.5). Stir the tube vigorously on a vortex mixer.
4. Leave the tubes to sit at **room temperature** for ~5 min and then stir the contents again. Filter the slurry through a Whatman No. 1 (9 cm) filter circle.
5. The absorbance of the reaction solutions are then measured at 590 nm against the **substrate/enzyme** blank

A **substrate/enzyme** blank is prepared by adding 10 ml of Tris buffer salt solution to the enzyme solution (0.5 mL) before the addition of the Arabinazyme tablet. The slurry must be left at **room temperature.**

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** from *Aspergillus niger* on linear 1,5-α-L-arabinan [carboxymethyl form] and Arabinazyme (Lot 250416) is shown in Figure 1. Enzyme 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.

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.

CALCULATION OF ACTIVITY:

endo-Arabinanase activity in the sample being assayed is determined by reference to the standard curve or to a regression equation for Arabinazyme tablets (Figure 1).

The regression equation varies slightly from batch to batch of Arabinazyme tablets. To minimise this inconvenience, large batches of tablets are prepared.

For Arabinazyme Lot 250416:

endo-Arabinanase milli-Units/assay (i.e. 0.5 mL)

$$= 14.5 \times Abs.^2 + 39.3 \times Abs.$$

endo-Arabinanase activity per mL or g of original preparation:

$$= \text{milli-Units/assay} \times 2 \times \frac{1}{1000} \times \text{Dilution}$$

where:

2 = conversion from 0.5 mL (as assayed) to 1.0 mL.

1/1000 = conversion from milliUnits to Units.

Dilution = total dilution of the original enzyme preparation.

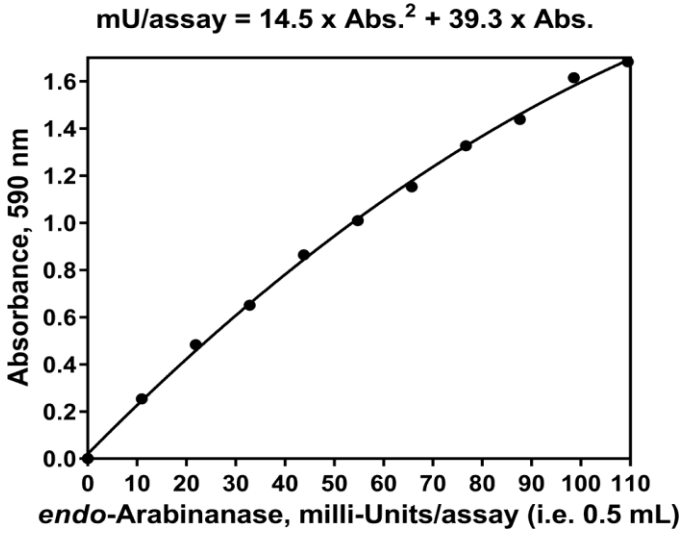

Figure 1. *endo*-Arabinanase (*A. niger*) standard curve on Arabinazyme tablets (Lot 250416).

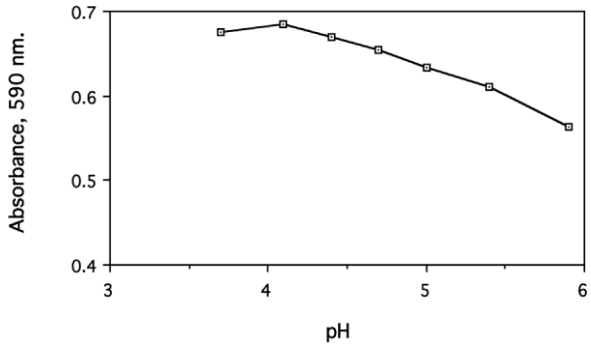

Figure 2. Effect of pH on the activity of *endo*-arabinanase on Arabinazyme tablets.

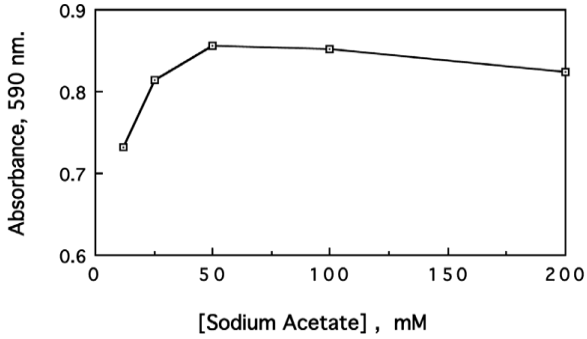

Figure 3. Effect of buffer salt concentration on the activity of *endo*-arabinanase on Arabinazyme tablets.

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