Megazyme

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ASSAY OF

endo-1,4-ß-D-GALACTANASE

using

GALACTAZYME TABLETS

T-GLZ 02/14

SUBSTRATE:

The substance employed in the Galactazyme tablets is azurine-crosslinked-potato galactan (AZCL-Galactan). Galactan is purified from potato fibre and is then incubated with *endo*-arabinanase and α-L-arabinofuranosidase to remove most of the α-linked L-arabinofuranose. This arabinose-reduced galactan polysaccharide typically contains galactose (> 88%), arabinose (~ 2%), rhamnose (~ 3%), and galacturonic acid (7%). This polysaccharide is then dyed and crosslinked.

The α-L-arabinose in potato pectin-galactan occurs as short chains and is slightly susceptible to hydrolysis by *endo*-arabinanase. Incubation of this pectic-galactan with α-L-arabinofuranosidase reduces the arabinose content to less than 2%. This polysaccharide, when dyed and crosslinked, is not hydrolysed by *endo*-arabinanase, or polygalacturonanase, demonstrating that the substrate is specific for the measurement of *endo*-β-1,4-galactanase in crude enzyme mixtures. The *exo*-acting enzyme α-L-arabinofuranosidase can release traces of arabinose from the dyed, crosslinked substrate, but it is unable to release dyed fragments. Consequently, it does not interfere in the assay.

BUFFER:
(Sodium Acetate buffer, 100 mM, pH 4.0) containing sodium azide (0.02%) and BSA (0.5 mg/mL).

Add 6.1 g of glacial acetic acid (1.05 g/mL) to 900 mL of distilled water. Adjust the pH to 4.0 by addition of 1 M (4 g/100 mL) sodium hydroxide solution. Approximately 20 mL is required. Then add 0.2 g of sodium azide and 0.5 g of BSA. Adjust the volume to 1 L with distilled water and store in a sealed Duran bottle at 4°

NOTE: Addition of sodium azide (a preservative) is optional.

Do not add the sodium azide until the pH is adjusted. Acidification of sodium azide releases a poisonous gas.

ENZYME DILUTION:

Liquid preparations:

With a positive displacement dispenser, add 1.0 mL of liquid enzyme preparation to 99.0 mL of sodium acetate buffer (100 mM, pH 4.0) containing sodium azide plus BSA and stir well on a vortex mixer. Dilute this solution further by adding 1.0 mL of the enzyme solution to 9.0 mL of sodium acetate buffer (100 mM, pH 4.0). Repeat this operation until a dilution suitable for assay is achieved.

Powder samples:

Add 1.0 g of powder preparation to 100 mL of sodium acetate buffer (100 mM, pH 4.0) containing sodium azide plus BSA and mix well on a magnetic stirrer until completely dissolved or dispersed (approx. 10 min). Clarify the solution by centrifugation (1,500 g x 10 min) or by filtration through Whatman No. 1, 9 cm filter circles. Dilute this solution further by adding 1.0 mL of the enzyme solution to 9.0 mL of sodium acetate buffer (100 mM, pH 4.0). Repeat this operation until a dilution suitable for assay is achieved. If reaction absorbance values are greater than **1.6**, dilute the enzyme solution and repeat the assay.

ASSAY PROCEDURE

1. Pre-equilibrate an aliquot (0.5 mL) of suitably diluted enzyme preparation in sodium acetate buffer (100 mM, pH 4.0) containing sodium azide and BSA at 40°C for 5 min.
2. Initiate the reaction by adding a Galactazyme tablet. The tablet hydrates rapidly. Do not stir the suspension. Incubate at 40°C for exactly 10 min.
3. Terminate the reaction by adding 10.0 mL of Trizma Base solution (2% w/v, pH ~ 10) with vigorous stirring on a vortex mixer.
4. Leave the tubes at room temperature for about 5 min, and then stir them again.
5. Filter the slurry through a Whatman No. 1 (9 cm) filter circle.
6. Measure the absorbance of the filtrate at 590 nm against a substrate blank.

If the absorbance is above 1.5, dilute an aliquot of the enzyme extract with an equal volume of extraction/dilution buffer and repeat the assay.

A substrate/enzyme blank is prepared by adding Trizma Base to the enzyme solution before the addition of the Galactazyme tablet.

> **NOTE:** A single blank is required for each set of determinations and this is used to zero the spectrophotometer. The absorbance of the reaction solutions is then measured at 590 nm against the reaction blank.

STANDARDISATION:

A **standard curve** relating the activity of purified *endo*-galactanase on potato galactan (~ 88% galactose) and Galactazyme (lot 50802) is shown in Figure 1. Activity on potato galactan (Lot 120501) was determined using *endo*-galactanase in 100 mM sodium acetate buffer (pH 4.0) containing BSA (0.5 mg/mL) and sodium azide (0.02%) on potato galactan at 10 mg/mL in 100 mM sodium acetate buffer (pH 4.0) and 40°C using the Nelson/Somogyi reducing sugar procedure. The curves obtained for *endo*-galactanase in the crude commercial enzyme preparations tested, were the same.
One **Unit** of activity is defined as the amount of enzyme required to release one micromole of galactose reducing-sugar equivalents from potato galactan per minute under the defined assay conditions.

CALCULATION OF ACTIVITY:

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

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

For Galactazyme Lot 50802:

endo-Galactanase, mU/assay (i.e. /0.5 mL)
= 120.3 x Absorbance

endo-Galactanase, U/per mL or gram of original preparation:

$$= \text{mU/assay} \times \frac{2}{1000} \times \text{Dilution}$$

where:

$\frac{2}{1000}$ = conversion from milliUnits/0.5ml to Units/mL

Dilution = total dilution of the original enzyme preparation.

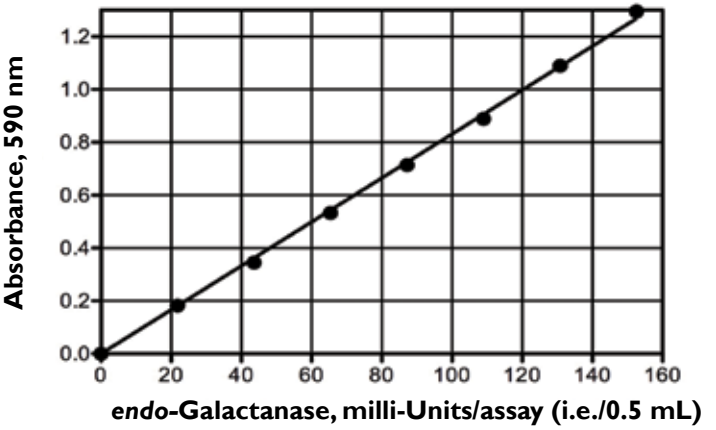

Figure 1. *endo*-Galactanase standard curve on Galactazyme tablets (Lot 50802)

endo-Galactanase (0.5 mL, 0-152 milliUnits) in 100 mM sodium acetate buffer (pH 4.0) containing sodium azide (0.02%) and BSA (0.5 mg/mL) was pre-equilibrated at 40°C for 5 min. The reaction was initiated by the addition of a Galactazyme tablet ***without stirring.*** Reaction was terminated after 10 min by the addition of Tris Base (10 mL, 2% w/v, pH ~ 10) with vigorous stirring. After approx. 4-5 min, the tubes were stirred again, and the solutions were filtered through Whatman No. 1 (9 cm) filter circles, and the absorbance of the reaction solutions were read against an enzyme/substrate blank solution at 590 nm.

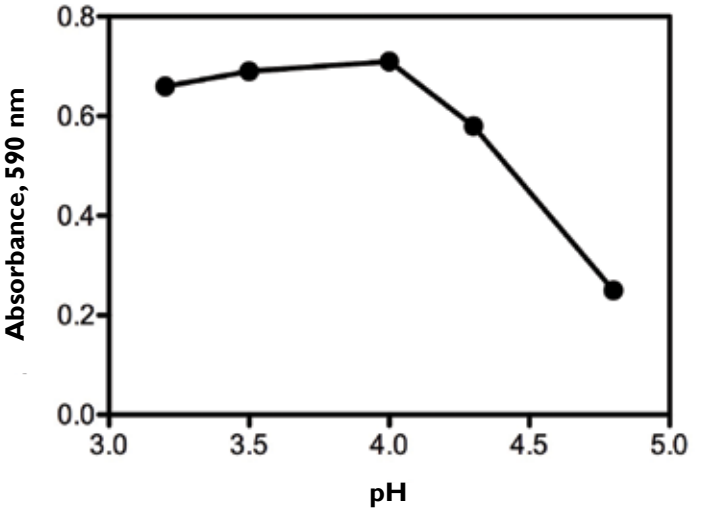

Figure 2. Effect of pH on the activity of *endo*-Galatanase on Galactazyme tablets

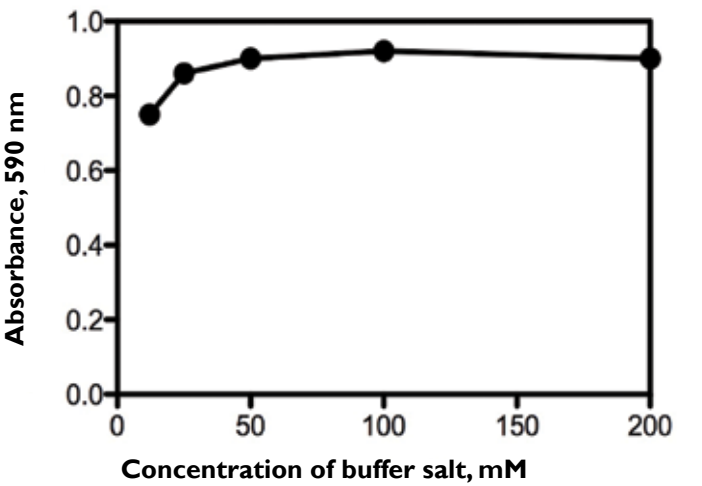

Figure 3. Effect of buffer salt concentration on the activity of *endo*-Galactanase on Galactazyme tablets

NOTES:

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