

Megazyme

ASSAY OF
endo-1,4- β -D-
GALACTANASE
using
GALACTAZYME
TABLETS



SUBSTRATE:

The substance employed is Azurine-crosslinked-potato galactan which has been treated to remove most of the arabinofuranosyl residues (AZCL-Galactan). Highly purified galactan is extracted from potato fibre. This is treated with α -L-arabinofuranosidase to remove arabinose. This arabinose-reduced polysaccharide typically contains galactose(>88%), arabinose(~2%), rhamnose(3%), and galacturonic acid(7%). The polysaccharide is then dyed and crosslinked.

The arabinose in potato pectin-galactan occurs as short chains and is slightly susceptible to hydrolysis by *endo*-arabinanase. However, treatment of this pectic-galactan with α -L-arabinofuranosidase reduces the arabinose content to less than 2%. When this polysaccharide was dyed and crosslinked, it was not hydrolysed by *endo*-arabinanase, or polygalacturonanase, demonstrating that the substrate is specific for the measurement of *endo*-1,4- β -D-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, 100mM, pH 4.0) containing sodium azide (0.02%).

Glacial acetic acid(6.1g, 1.05g/ml) is added to 900ml of distilled water. This solution is adjusted to pH 4.0 by addition of 1M (4g/100ml) sodium hydroxide solution. Approximately 20ml is required. Sodium azide (0.2g) is added and the volume is adjusted to 1 Litre. Store at 4°C.

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 enzyme preparation (1.0ml) is added, using a positive displacement dispenser, to sodium acetate buffer(9.0ml). This solution is then further serially diluted by addition of 1.0ml of sodium acetate buffer (9.0ml, 100mM, pH 4.0).

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

If reaction values greater than **1.6 Absorbance Units** are obtained, the enzyme solution should be diluted further in sodium acetate buffer (100mM, pH 4.0) and the assay repeated

ASSAY PROCEDURE:

Aliquots (0.5ml), suitably diluted enzyme preparation in acetate buffer (100mM, pH 4.0) are pre-equilibrated to 40°C for 5 min. **Reaction** is initiated by the addition of a Galactazyme tablet. The tablet hydrates rapidly. The suspension **should not** be stirred. After exactly 10 min at 40°C, the reaction is terminated by the addition of Trizma Base solution (10ml, 2%w/v, Sigma cat. no. T-1503) with vigorous stirring on a vortex mixer. After about 4-5 min standing at room temperature, the slurry is stirred again and then filtered through a Whatman No.1 (9 cm) filter circle.

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 are 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 50801) is shown in Figure 1. The curves obtained for endo-galactanase in the crude commercial enzyme preparations tested, were the same. Activity on potato galactan was determined at a substrate concentration of 5mg/ml in 100mM sodium acetate buffer (pH 4.0) at 40°C using the Nelson/Somogyi reducing sugar procedure. 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:

1. endo-Galactanase activity in the sample being assayed is determined by reference to the standard curve to convert absorbance values to milliUnits per assay (ie. per ml).

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

$$Y = MX + C.$$

WHERE:

Y= endo-galactanase activity (in milliUnits /assay, ie per 0.5 ml).

M= Slope of the calibration graph.

X= Absorbance of the reaction at 590nm (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 Galactazyme tablets. **M** and **C** values for the particular batch of tablets are provided with the tablets.

For Galactazyme Lot 50801:

milliUnits/assay (0.5ml)=166 x Absorbance -3.3

2. endo-Galactanase activity per **ml** or gram of original preparation:

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

WHERE:

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

Dilution = the dilution of the original enzyme preparation.

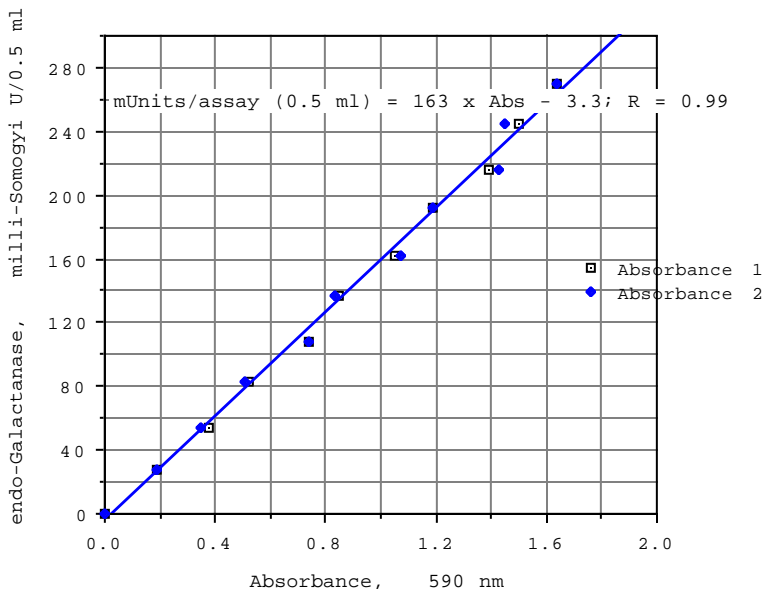


Figure 1. *endo*-Galactanase standard curve on Galactzyme tablets (Lot 50801)

endo-Galactanase (0.5ml, 0-270 milliUnits) in 100 mM sodium acetate buffer (pH 4.0) containing sodium azide (0.02%) was pre-equilibrated at 40°C for 5 min. The reaction was initiated by the addition of a **Galactzyme** tablet without stirring. Reaction was terminated after 10 min by the addition of Trizma Base (10ml, 2% w/v) with vigorous stirring. After about 4-5 min, the tubes were stirred again, and the solutions filtered through Whatman No. 1 (9cm) filter circles, and the absorbance of the reaction solutions were read against an enzyme/substrate blank solution at 590nm.

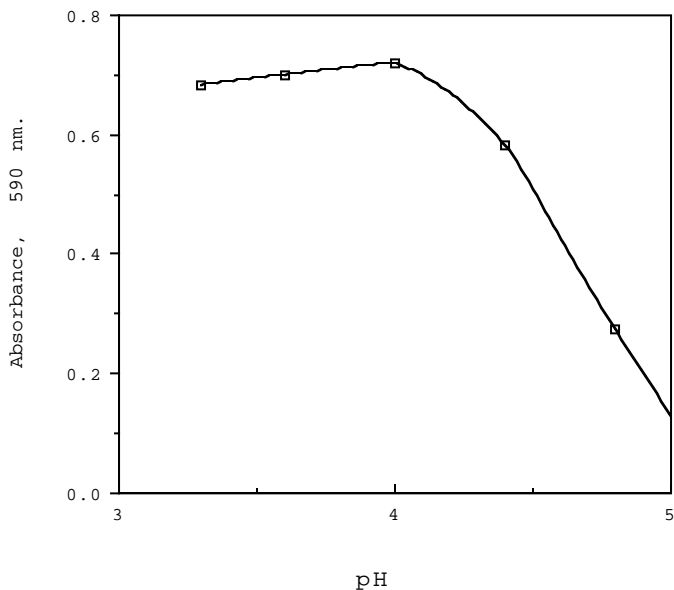


Figure 2. Effect of pH on the activity of *endo*-Galactanase on Galactazyme tablets

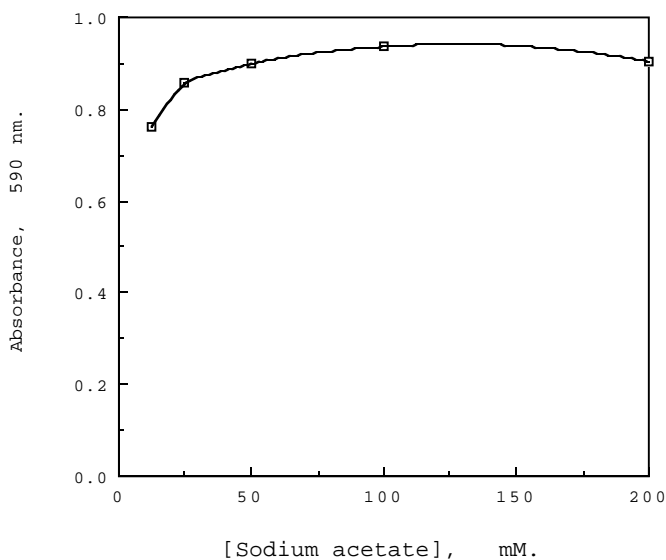


Figure 3. Effect of buffer salt concentration on the activity of *endo*-Galactanase on Galactazyme tablets



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