

Megazyme

ASSAY OF
endo-1,3-Beta-Glucanase
using
**1,3-BETA-GLUCAZYME
TABLETS**

PAZ 11/99



SUBSTRATE:

The substrate employed is Azurine-crosslinked pachyman (AZCL-Pachyman). The substrate is prepared by dyeing and crosslinking highly purified pachyman to produce a material which hydrates in water but is water insoluble. Hydrolysis by *endo*-1,3- β -glucanase produces water soluble dyed fragments, and the rate of release of these (increase in absorbance at 590 nm) can be related directly to enzyme activity. The substrate is supplied commercially in a ready-to-use tablet form as 1,3-Beta-Glucazyme Tablets.

STOCK BUFFER:

(Sodium acetate buffer, 2M, pH 4.5) Glacial acetic acid (114 ml, 1.05 g/ml) is added to 800 ml of distilled water. This solution is adjusted to pH 4.2 by the addition of 4 M (16 g/100 ml) sodium hydroxide solution. The volume is adjusted to 1 litre.

EXTRACTION/DILUTION BUFFER:

(Na acetate, 200 mM, pH 4.5, plus Na azide (0.02%).

Stock buffer (100 ml) is added to 850 ml of distilled water and sodium azide (0.2g) is added and dissolved. The pH is adjusted to pH 4.5 by dropwise addition of 1M hydrochloric acid solution. The volume is adjusted to 1 litre.

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

ENZYME EXTRACTION AND DILUTION:

- Add 0.50 g of malt flour (milled to pass a 0.5 mm screen to a glass centrifuge tube (16 x 120 mm).
- Add 5.0 ml of Extraction buffer solution (200 mM sodium acetate buffer, pH 4.5) and stir the contents thoroughly on a vortex mixer.
- Allow the enzyme to extract over a 15 min period at room temperature (less than 30°C), with occasional mixing.
- Centrifuge the tube and contents at 1,000g for 10 min, or filter the slurry through glass fibre filter paper (Whatman GF/C).

ASSAY PROCEDURE:

- An aliquot (1.0 ml) of suitably diluted and buffered enzyme preparation in a 16 x 120 mm glass test tubes is pre-equilibrated to 30°C for 5 min.

- Reaction is initiated by adding a 1,3-Beta-Gluczyme Tablet. The tablet hydrates rapidly. The suspension should not be stirred.
- After exactly 10.0 min (from addition of the tablet), the reaction is terminated by the addition of Trizma Base solution (10.0 ml, 2% w/v, Sigma Cat. No. T-1503) with vigorous stirring on a vortex mixer.
- The tubes are left at room temperature for about 5 min, the slurry is stirred again, and it is then filtered through a Whatman No.1 (9 cm) filter circle.
- The absorbance of the filtrate is measured at 590nm against a substrate blank.

The substrate blank is prepared by adding a 1,3-Beta-Gluczyme tablet to 1.0 ml of extraction buffer, incubating at 30°C for 10 min, adding 10.0 ml of Trizma Base (2% w/v) and filtering after 5 min.

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 measured at 590 nm against the blank. If the absorbance is above 1.4, dilute an aliquot of the enzyme extract with an equal volume of extraction/dilution buffer and repeat the assay.

STANDARDISATION:

A standard curve relating the activity of purified malt 1,3-B-glucanase on 1,3-Beta-Gluczyme (Lot 30102) is shown in the attached graph (Figure 1). This curve is not linear. Enzyme activity was first standardised on CM-Pachyman (DS=0.1) at substrate concentration of 10 mg/ml in 100 mM sodium acetate buffer (pH 4.5) at 30°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 reducing-sugar equivalents per minute under the defined assay conditions.

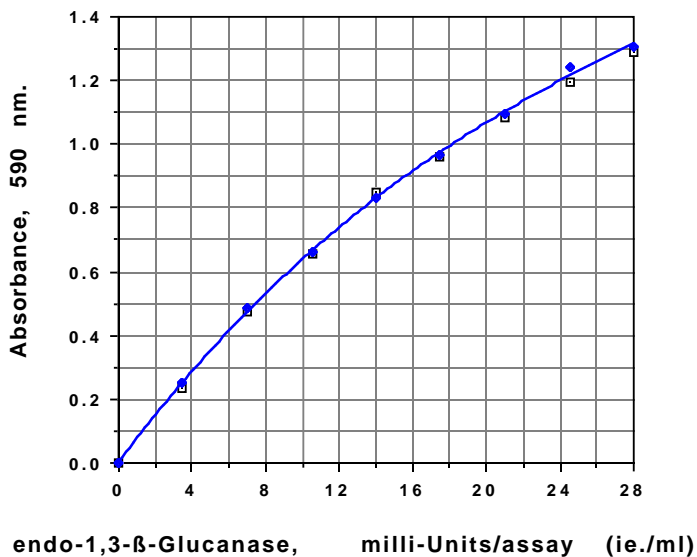


Figure 1. *endo*-1,3-Beta-glucanase standard curve on 1,3-Beta-Gluczyme tablets (Lot 30102).

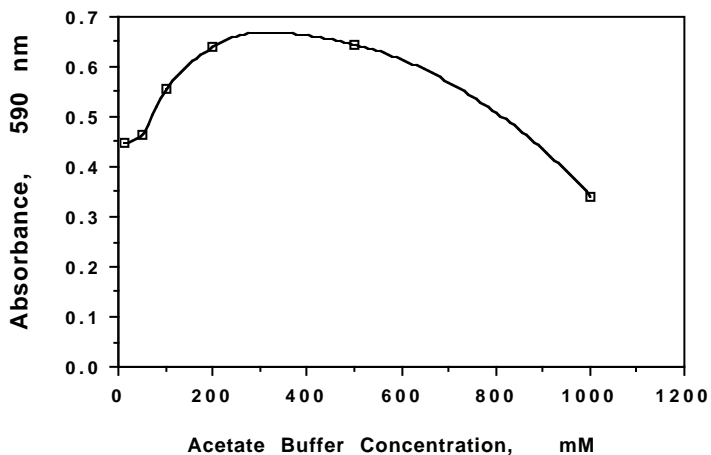


Figure 2. Effect of buffer salt concentration on the sensitivity of the 1,3-Beta-Gluczyme test.



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