

Megazyme

ASSAY OF
endo-1,4- β -Mannanase
using

**BETA-MANNAZYME
TABLETS**

TMNZ 1/02



SUBSTRATE:

The substrate employed is Azurine-crosslinked carob galactomannan (AZCL-Galactomannan). The substrate is prepared by dyeing and crosslinking highly purified carob galactomannan to produce a material which hydrates in water but is water insoluble. Hydrolysis by *endo*-1,4- β -mannanase 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 **Beta-Mannazyme** Tablets.

STOCK BUFFER:

(Sodium acetate buffer, 2M, pH 4.0)

Glacial acetic acid (114 ml, 1.05 g/ml) is added to 800 ml of distilled water. This solution is adjusted to pH 4.0 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, 50 mM, pH 4.5, plus Na azide (0.02%)).

Stock buffer (25 ml) is added to 950 ml of distilled water and the pH is adjusted to pH 4.0 by dropwise addition of 1M hydrochloric acid solution. Sodium azide (0.2g) is added and dissolved. 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 1.0 g of powder enzyme preparation, or 1.0 ml of liquid enzyme preparation (using a positive displacement pipetter), to a 100 ml volumetric flask, adjust to volume with extraction buffer (50 mM sodium acetate buffer, pH 4.0) and stir on a magnetic stirrer for 15 min. Ensure that the preparation is completely dissolved or dispersed. (This is referred to as the **Original Enzyme Extract**). Filter an aliquot of the slurry through a Whatman No. 1 (9 cm) filter circle. Dilute extracts by adding 1.0 ml of enzyme solution in 9.0 ml of dilution buffer with thorough mixing. Repeat this operation to obtain the correct enzyme concentration for assay. (For Gamanase from Novozymes, an overall dilution of the original liquid solution of 50-fold is required).

ASSAY PROCEDURE:

Pre-equilibrate an aliquot (0.5 ml) of suitably diluted and buffered enzyme preparation in a 16 x 120 mm glass test tubes at 40°C for 5 min. Initiate reaction by adding a Beta-Mannazyme tablet. (The tablet hydrates rapidly-do not stir the tubes).

Terminate the reaction exactly 10.0 min after addition of the tablet by adding 10.0 ml of Trizma Base solution (2% w/v, pH ~ 8.5, Sigma Cat. No. T-1503) with vigorous stirring on a vortex mixer. Leave the tubes at room temperature for about 5 min, and then stir them again. Filter the tube contents through a Whatman No. 1 (9 cm) filter circle.

Prepare a substrate blank by adding a Beta-Mannazyme tablet to 0.5 ml of extraction buffer. Incubate at 40°C for 10 min, add 10.0 ml of Trizma Base (2% w/v) and filter after 5 min.

NOTE:

1. 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.
2. After the Trizma base solution is added, the suspensions must be stored at room temperature. If stored at higher temperatures, the substrate will slowly degrade in the alkaline conditions resulting in higher blank absorbance values.

STANDARDISATION:

A standard curve relating the activity of purified *A. niger* 1,4- β -mannanase on Beta-Mannazyme (Lot 50201) is shown in the attached graph (Figure 1). This curve is relatively linear. Enzyme activity was first standardised on carob galactomannan at substrate concentration of 10 mg/ml in 100 mM 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 mannose reducing-sugar equivalents per minute under the defined assay conditions.

CALCULATION OF ACTIVITY:

1. *endo*- β -Mannanase activity in the sample being assayed is determined by reference to the standard curve to convert absorbance values to **milli-Units per assay (ie. per 0.5 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*- β -Mannanase 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 Beta-Mannazyme tablets. **M** and **C** values for the particular batch of tablets are provided with the tablets.

For Beta-Mannazyme Lot 50201:

$$\text{milliUnits/assay (0.5ml)} = 139 \times \text{Absorbance} - 5.7$$

2. *endo*- β -Mannanase activity per **ml or g** of original preparation:
= Y x 100/1.0 x 2 x 1/1000 x Further dilution

Where:

- Y = *endo*- β -Mannanase activity (in milliUnits /assay, ie per 0.5 ml).
- 100/1.0 = 1 g or ml of enzyme extracted in 100 ml of buffer.
- 2 = conversion from 0.5 ml (as assayed) to 1.0 ml.
- 1/1000 = conversion from milliUnits to Units.
- Further dilution = further dilution of the original enzyme extract.

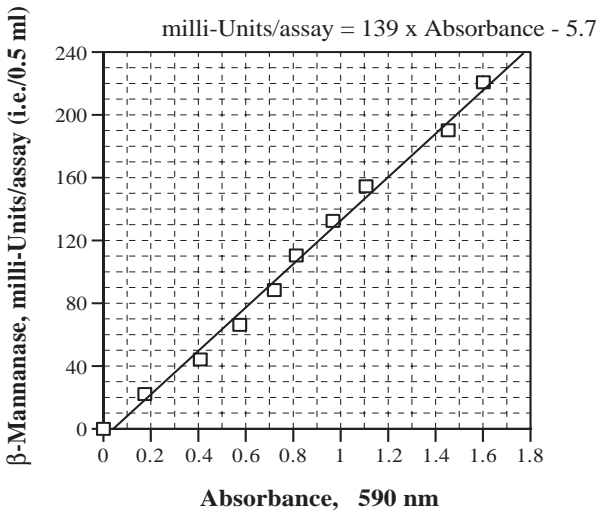


Figure 1. *endo*-1,4- β -mannanase standard curve on Beta-Mannazyme tablets (Lot 50201) at pH 4.0.



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