Megazyme

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ASSAY OF

endo-1,3-β-Glucanase

using

1,3-β-GLUCAZYME TABLETS

T-PAZ-200T 03/16

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-β-Glucazyme Tablets.

STOCK BUFFER:
(Sodium acetate buffer, 2 M, pH 4.5)

Add 114 mL of glacial acetic acid (1.05 g/mL) to 800 mL of distilled water. This solution is adjusted to pH 4.5 by the addition of 4 M (16 g/100 mL) sodium hydroxide solution. The volume is adjusted to 1 L.

EXTRACTION/DILUTION BUFFER:
(Sodium acetate, 200 mM, pH 4.5, plus sodium azide 0.02%).

Add 100 mL of stock buffer solution to 850 mL of distilled water and adjust the pH to 4.5. Add 0.2 g of sodium azide and dissolve. Adjust the volume to 1 L. Store at 4°C.

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

STOPPING REAGENT:

Tris Buffer Salt Solution (2% w/v; pH ~ 10)

Dissolve 20 g of tris buffer salt (Megazyme Cat. no. **B-TRIS500**) in 1 L of distilled water. Store this solution at room temperature.

ENZYME EXTRACTION AND DILUTION:

A. Cereal Flours and Malt Extracts:

Add 0.50 g of milled malt (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 15 min at room temperature (less than 30°C), with occasional mixing. Centrifuge the tube and contents at 1,000 *g* for 10 min or filter the slurry through glass fibre filter paper (e.g. Whatman GF/C).

B. Microbial Enzyme Preparations:

Using a positive displacement dispenser, add 1.0 mL of liquid enzyme preparation to a 100 mL volumetric flask and dilute to volume with 200 mM sodium acetate buffer (pH 4.5), and mix thoroughly. Further dilute this solution by adding 1.0 mL to 9.0 mL of buffer with thorough mixing. Dilute further as required for assay.

Add 1.0 g of powder sample to 90 mL of 200 mM sodium acetate buffer (pH 4.5) and mix until either completely dissolved or dispersed, and adjust to 100 mL. Clarify an aliquot of this solution by centrifugation (1,000 *g*, 10 min) or filtration through a Whatman No. 1 filter circle. Dilute further in 200 mM sodium acetate buffer, as required for assay.

ASSAY PROCEDURE:

A. 1,3-β-Glucanase in Cereal Flours and Malt Extracts:

1. Pre-equilibrate an aliquot (0.5 mL) of suitably diluted and buffered enzyme preparation in a 16 x 120 mm glass test tube at 30°C for 5 min.
2. Add a 1,3-β-Glucazyme tablet to the tube without stirring. The tablet hydrates rapidly. Incubate at 30°C for exactly 10 min.
3. Terminate the reaction by adding 10.0 mL of Tris Buffer Salt 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.

The **substrate blank** is prepared by adding a 1,3-β-Glucazyme tablet to 0.5 mL of extraction buffer, incubating at 30°C for 10 min, adding 10.0 mL of Tris Buffer Salt Solution (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 1.6, 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 *endo*-1,3-β-Glucanase from *Trichoderma sp.* on 1,3-β-Glucazyme (Lot 11201) is shown in the attached graph (Figure 1). This curve is not linear. Enzyme activity was first standardised on CM-Pachyman (DS = 0.2) at substrate concentration of 10 mg/mL in 200 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

CALCULATION OF ACTIVITY:

Endo-1,3-β-Glucanase activity in the sample being assayed is determined by reference to the appropriate standard curve or regression equation to convert absorbance (590 nm) into milli-Units of activity per assay.

The regression equation varies slightly from batch to batch of 1,3-β-Glucazyme tablets. To minimise this inconvenience, large batches of tablets are prepared.

Units/mL or gram of original preparation:

$$= \text{milli-Units (per assay, i.e. per 0.5 mL)} \times \frac{1}{1000} \times 2 \times \text{Initial extract} \times \text{Dilution}$$

where:

$\frac{1}{1000}$	=	conversion from milli-Units to Units.
2	=	conversion from volume assayed (0.5 mL) to 1 mL of extract.
Initial extract	=	10 for cereal flours (0.5 g in 5 mL)
	=	100 for microbial enzyme preparations (1 mL or 1 g in 100 mL)
Dilution	=	further dilution of the initial extraction solution.

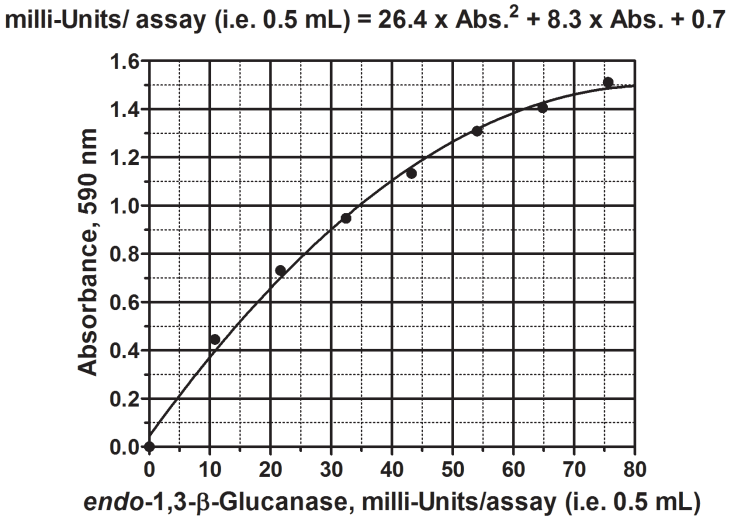

Figure 1. *endo*-1,3-β-Glucanase (*Trichoderma sp.)* standard curve on 1,3-β-Glucazyme tablets (Lot 11201).

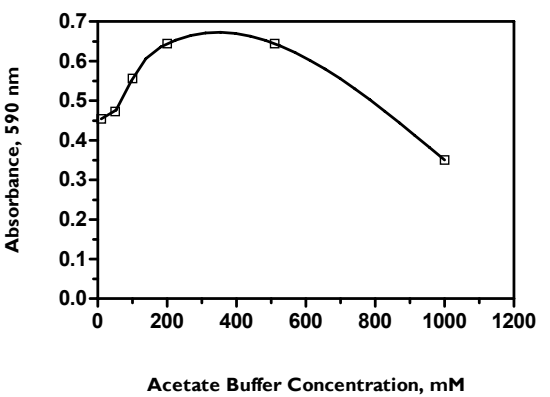

Figure 2. Effect of buffer salt concentration on the sensitivity of the 1,3-β-Glucazyme test.

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