Megazyme

endo-1,4-ß-GLUCANASE

(Cellulase)

ASSAY PROCEDURE

CELLAZYME AF TABLETS

T-CAF1000 08/13

Megazyme

SUBSTRATE:

The substrate employed is azurine-crosslinked HE-cellulose (AZCL-Cellulose). It is prepared by dyeing and crosslinking HE-cellulose to produce a material which hydrates in water but is water insoluble. Hydrolysis by *endo*-1,4-ß-D-glucanase (cellulase) 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. In this technical booklet, the use of Cellazyme AF tablets is described. These tablets are identical to Cellazyme C tablets except that they are just 40 mg, compared to Cellazyme C, which are 60 mg. This smaller size allows more economical use of the substrate in larger scale screening. Repeatability with these tablets is slightly reduced as a result of the lower amount of substrate per test.

BUFFER STOCK SOLUTION: (Sodium Acetate buffer, 1 M, pH 4.5)

Add 60.0 g of glacial acetic acid (1.05 g/mL) to 800 mL of distilled water. Adjust the pH of this solution to pH 4.5 by the addition of 5 M (20 g/100 mL) NaOH solution. Adjust the volume to 1 litre. Stable at room temperature for several months.

EXTRACTION/DILUTION BUFFER:

[Sodium acetate, 25 mM, pH 4.5 containing sodium azide (0.02%)]

Add 25 mL of buffer stock solution to 850 mL of distilled water. Adjust the pH to pH 4.5 by dropwise addition of 2 M hydrochloric acid. Add 0.2 g of sodium azide, dissolve, and adjust the volume to 1 litre.

ENZYME EXTRACTION AND DILUTION:

Add 1.0 mL of liquid enzyme preparation to 49 mL of Extraction/Dilution buffer (pH 4.5) using a positive displacement dispenser (these solutions can be very viscous), and mix thoroughly. This is termed the **Original Extract**. Add 1.0 mL of this solution to 9.0 mL of

NOTES:

1. When preparing the extraction buffer, do not add the sodium azide until the pH is adjusted. Acidification of sodium azide releases a poisonous gas.
2. In the assay format described here, **a single blank** is required for each set of determinations and this is used to zero the spectrophotometer. The absorbances of the reaction solutions are read against this blank.
3. Stirring of the test tubes on addition of the Cellazyme AF tablet to the enzyme solution gives only a slight (about 5%) increase in the absorbance value, but the results are less reproducible.

Extraction/Dilution buffer (10-fold dilution). This process of dilution is repeated until a concentration suitable for assay is achieved.

With powdered samples, add 1.0 g of the preparation to 50 mL of Extraction/Dilution buffer (pH 4.5) and gently stir the slurry over a period of about 15 min or until the sample is completely dispersed or dissolved. Clarify this solution (the **Original Extract**) by centrifugation (1,000 g, 10 min) or filtration through Whatman No. 1 (9 cm) filter circles. Dilute this extract as required with the Extraction/Dilution buffer, as for the liquid enzyme preparations.

ASSAY PROCEDURE:

1. Pre-equilibrate 0.5 mL aliquots of suitably diluted enzyme preparation in sodium acetate buffer (25 mM, pH 4.5) at 40°C for 5 min in glass test tubes (16 x 120 mm).
2. Initiate the reaction by adding a Cellazyme AF tablet to the tube containing pre-equilibrated enzyme. The tablet hydrates rapidly. Do not stir the suspension.
3. Terminate the reaction after exactly 10 min at 40°C by adding 10.0 mL of Trizma Base solution (2% w/v, Sigma cat. no. T-1503) with vigorous stirring on a vortex mixer.
4. Allow the tubes to stand for approx. 4-5 min at room temperature, and then stir the contents again. Filter the slurry through a Whatman No. 1 (9 cm) filter circle.
5. Measure the absorbance of the filtrate at 590 nm against a **substrate/enzyme blank**. The **substrate/enzyme blank** is prepared by adding Trizma Base to the enzyme solution before the addition of the Cellazyme AF tablet. This slurry **must** be left at room temperature. A single blank is required for each set of determinations and this is used to zero the spectrophotometer.

STANDARDISATION:

A **standard curve** relating the activity of pure *Trichoderma* sp. *endo*-cellulase (*endo*-glucanase) from the commercial enzyme preparation Laminex BG (from Genencor International) on CM-Cellulose 4M (CMC-4M) and Cellazyme AF Tablets (Lot 100901) is shown in Figure 1. Activity on CMC-4M was determined at a substrate concentration of 10 mg/mL in 100 mM sodium acetate buffer (pH 4.5) at 40°C using the Nelson/Somogyi reducing sugar method. The effects of pH and buffer salt concentration on activity are shown in Figures 2 and 3.

One **Unit** of activity is defined as the amount of enzyme required

to release one µmole of D-glucose reducing-sugar-equivalents per minute from CMC-4M (Somogyi reducing sugar method) at pH 4.5 and 40°C.

CALCULATION OF ACTIVITY:

***endo*-Cellulase** activity is determined by reference to the standard curve to convert absorbance to milliUnits of activity per assay on CMC-4M, and then calculated as follows:

Units/mL or gram of Original Preparation:

$$= \text{milliUnits (per assay, i.e. per 0.5 mL)} \times 2 \times 50 \times \frac{1}{1000} \times \text{Dilution}$$

where:

2 = conversion from 0.5 mL to 1.0 mL.

50 = the volume of buffer used to extract the original preparation (i.e. 1 g/50 mL or 1.0 mL of enzyme added to 49 mL buffer).

$\frac{1}{1000}$ = conversion from milliUnits to Units.

Dilution = further dilution of the Original Extract.

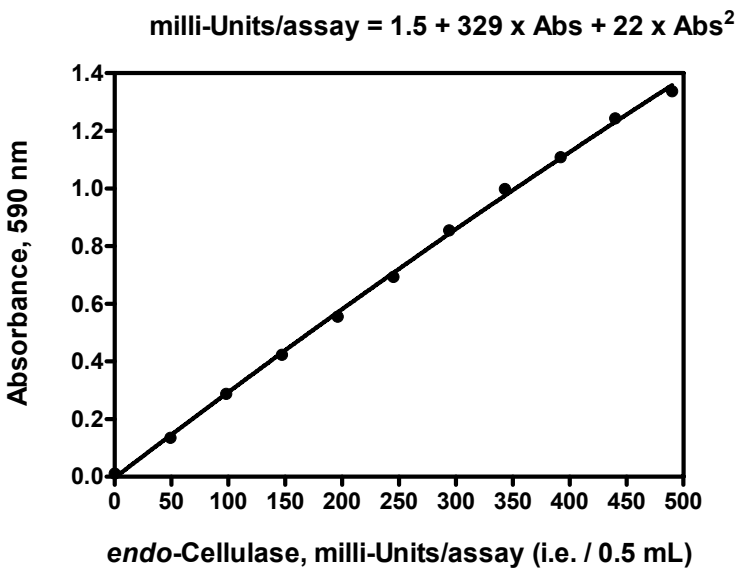

Figure 1. *Trichoderma* sp. *endo*-cellulase standard curve on Cellazyme AF (Lot 100901).

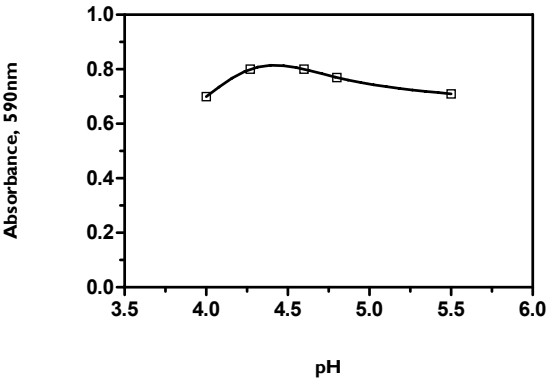

Figure 2. Effect of pH on the activity of *Trichoderma* sp. *endo*-cellulase on Cellazyme AF tablets.

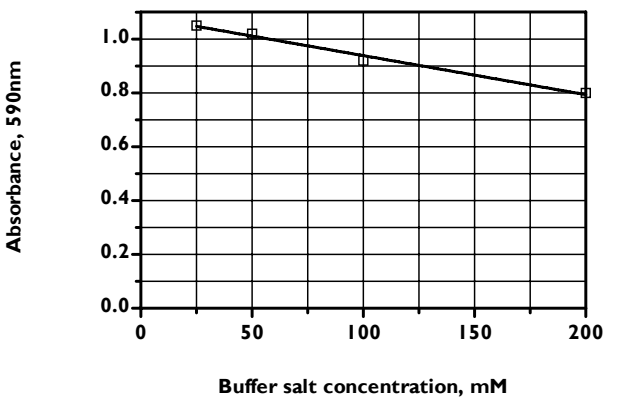

Figure 3. Effect of buffer salt concentration on the activity of *endo*-cellulase on Cellazyme AF tablet substrate.

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