Megazyme

www.megazyme.com

ASSAY OF

endo-CELLULASE

using

CELLAZYME C TABLETS

T-CCZ 01/17

SUBSTRATE:

The substrate employed is azurine-crosslinked HE-cellulose (AZCL-Cellulose). This substrate 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-β-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. The substrate is supplied commercially in a ready-to-use tablet form as **Cellazyme C** tablets (containing AZCL-HE-Cellulose).

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 L. 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 L.

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 C tablet to the enzyme solution gives only a slight (about 5%) increase in the absorbance value, but the results are less reproducible.

ENZYME EXTRACTION AND DILUTION:

Add 1.0 mL of liquid enzyme preparation to 49.0 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 Extraction/Dilution buffer (10-fold dilution). This process of dilution is repeated until a concentration suitable for assay is achieved. For example, for the industrial enzyme preparations, Finizym (from *Aspergillus niger*; Novo Nordisk, Denmark) and Laminex BG (from *Trichoderma* sp.; Genencor International, U.S.A.) a dilution of the original extract of approx. 200-fold is required.

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 C 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 2% Tris buffer salt solution (Megazyme cat. no. **B-TRIS500**) 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 Tris buffer to the enzyme solution before the addition of the Cellazyme C 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 *endo*-cellulase from *Trichoderma longibrachiatum* on CM-Cellulose (Megazyme cat. no. **P-CMC4M**) and Cellazyme C Tablets is shown below. Activity on CMC-4M was determined at a substrate concentration of 10 mg/mL in 100 mM sodium acetate buffer (pH 4.5) containing 0.5 mg/mL BSA 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

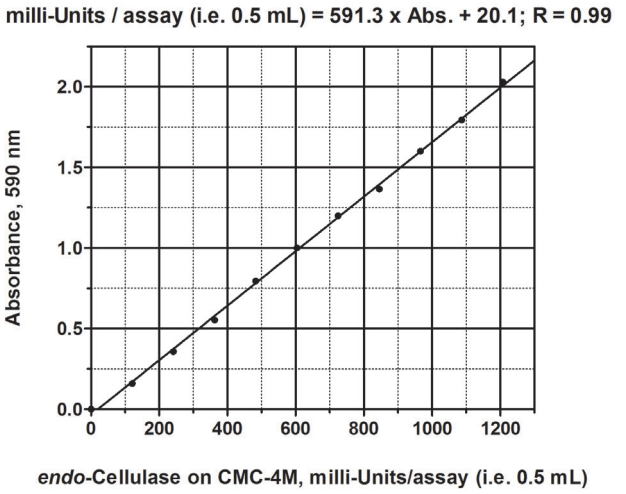

Figure 1. Standard curve relating activity of *endo*-cellulase from *Trichoderma longibrachiatum* (**E-CELTR**) on Cellazyme C (Lot 141001) at 40°C and pH 4.5 to activity on CMC-4M at 40°C and pH 4.5

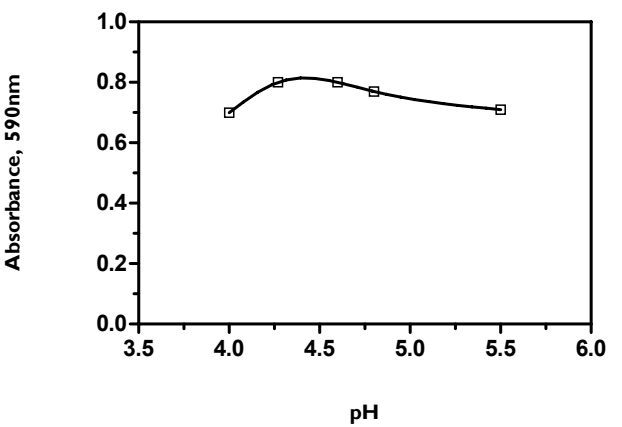

Figure 2. Effect of pH on the activity of *Trichoderma* sp. *endo*-cellulase on Cellazyme C tablets.

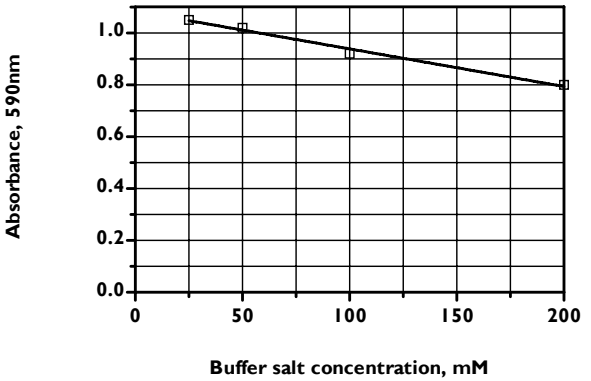

Figure 3. Effect of buffer salt concentration on the activity of *endo*-cellulase on Cellazyme C tablet substrate.

NOTES:

NOTES:

Bray Business Park, Bray,
Co. Wicklow,
A98 YV29,
IRELAND.

Telephone: (353.1) 286 1220
Facsimile: (353.1) 286 1264
Internet: www.megazyme.com
E-Mail: info@megazyme.com