CELLAZYME C TABLETS

PRODUCT INSTRUCTIONS

SKU: 700005100
T-CCZ

01/25

For the assay of *endo*-CELLULASE

200 Tablets / 1000 Tablets

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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:

Concentrated Acetate Buffer [Sodium acetate (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. Store at room temperature.

EXTRACTION/DILUTION BUFFER:

[Sodium acetate (25 mM, pH 4.7)]

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. Adjust the volume to 1 L. Store at room temperature.

STOPPING REAGENT:

[Tris Buffer Salt Solution (2% w/v; pH 9.0)]

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

NOTES:

1. In the assay format described here, **a single substrate/enzyme 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.
2. 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.

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 Tris buffer salt solution (2% w/v, pH ~ 9.0) with vigorous stirring on a vortex mixer.
4. Allow the tubes to stand for approx. 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 and Cellazyme C Tablets 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) 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 enzyme 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) under standard assay conditions (pH 4.5 and 40°C).

CALCULATION OF ACTIVITY:

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

Units/mL or gram of original preparation:

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

where:

$\frac{1}{1000}$ = conversion from milli-Units to Units

50 = initial extraction volume (i.e. 1 g/50 mL or 1.0 mL of enzyme added to 49 mL buffer).

2 = conversion from volume assayed (0.5 mL) to 1 mL of extract.

Dilution = further dilution of the initial extraction solution

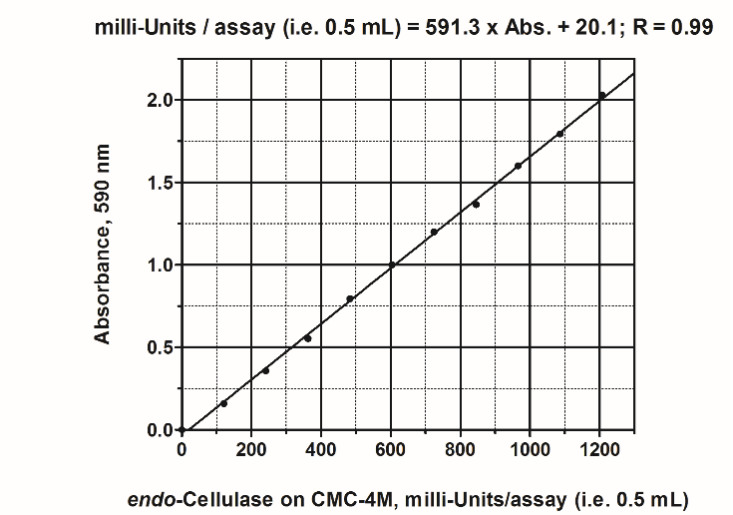

Figure 1. Standard curve relating activity of *endo*-cellulase from *Trichoderma longibrachiatum* on Cellazyme C (Lot 2410100 and 2410101) at 40°C and pH 4.5 to activity on CMC-4M at 40°C and pH 4.5.

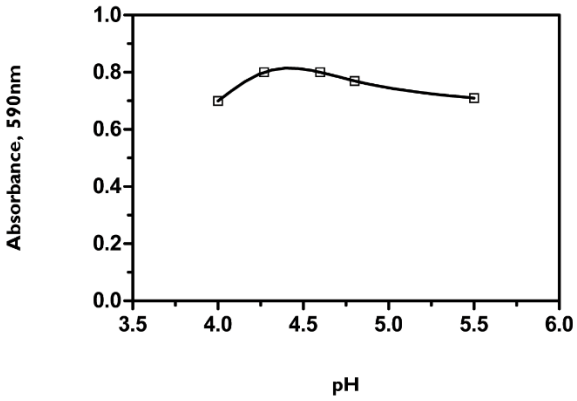

Figure 2. Effect of pH on the activity of *Trichoderma* sp. *endo*-cellulase on Cellazyme C tablets.

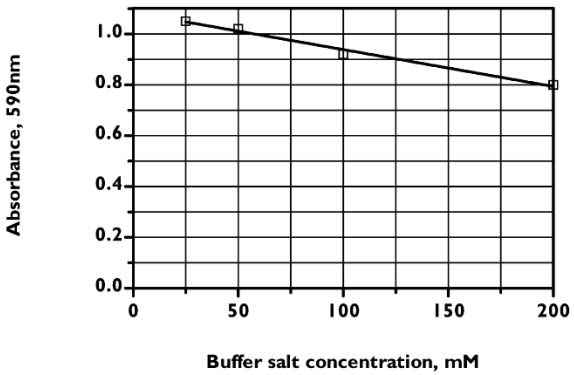

Figure 3. Effect of buffer salt concentration on the activity of *endo*-cellulase on Cellazyme C tablet substrate.

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