

Megazyme

ASSAY OF
endo-CELLULASE
USING
CELLAZYME C TABLETS

CZC 6/03



SUBSTRATE:

The substrate employed is azurine-crosslinked HE-cellulose (AZCL-Cellulose). The 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)

Glacial acetic acid (60.0 g, 1.05 g/ml) is added to 800 ml of distilled water. This solution is adjusted to pH 4.5 by the addition of 5 M (20 g/100 ml) sodium hydroxide solution. The volume is then adjusted to 1 litre. Store at room temperature.

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

Buffer stock solution (25 ml) is added to 850 ml of distilled water. The pH is adjusted to pH 4.5 by dropwise addition of 2 M hydrochloric acid solution. Sodium azide (0.2 g) is added and dissolved, and the volume is adjusted to 1 litre.

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:

Liquid enzyme sample (1.0 ml) is added, using a positive displacement dispenser (these solutions can be very viscous), to **Extraction/Dilution buffer** (49 ml, pH 4.5) and mixed thoroughly. This is termed the **Original Extract**. An aliquot of this solution (1.0 ml) is then diluted 10-fold by addition to 9 ml of **Extraction/Dilution buffer**. This process of dilution is repeated until a suitable dilution of the enzyme preparation 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 approximately 200-fold is required.

With powder samples, the preparation (1.0 g) is added to **Extraction/Dilution buffer** (50 ml, pH 4.5) and the slurry is gently mixed over a period of about 15 min or until the sample is completely dispersed or dissolved. This solution (the **Original Extract**) is clarified by centrifugation (1,000 *g*, 10 min) or filtration through Whatman No. 1 (9 cm) filter circles. This extract is then diluted further with **Extraction/Dilution buffer**, as for the liquid enzyme samples.

ASSAY PROCEDURE:

1. Aliquots (0.5 ml) of suitably diluted enzyme preparation in sodium acetate buffer (25 mM, pH 4.5) are pre-equilibrated to 40°C for 5 min in glass test tubes (16 x 120 mm).
2. **Reaction** is initiated by the addition of a Cellzyme C tablet. The tablet hydrates rapidly. The suspension **should not** be stirred.
3. After exactly 10 min at 40°C, the reaction is terminated by the addition of Trizma Base solution (10.0 ml, 2% w/v, Sigma cat.no. T-1503) with vigorous stirring on a vortex mixer.
4. After approximately 4-5 min standing at room temperature, the slurry is stirred again and then filtered 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 Cellzyme 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 *Trichoderma* sp. *endo*-cellulase (*endo*-glucanase) from the commercial enzyme preparation Laminex BG (from Genencor International) on CM-Cellulose 4M (CMC-4M) and Cellazyme C Tablets (Lot 80201) 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 micromole of 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 (about 200-fold for Laminex from Genencor International).

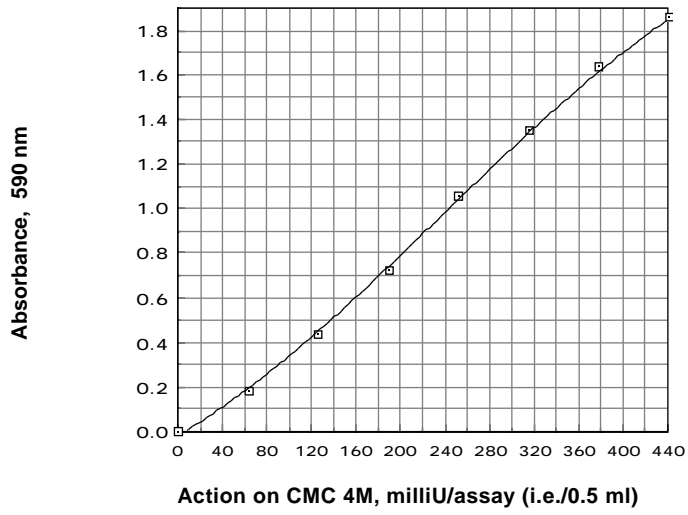


Figure 1. *Trichoderma* sp. endo-cellulase standard curve on Cellazyme C (Lot 80201).

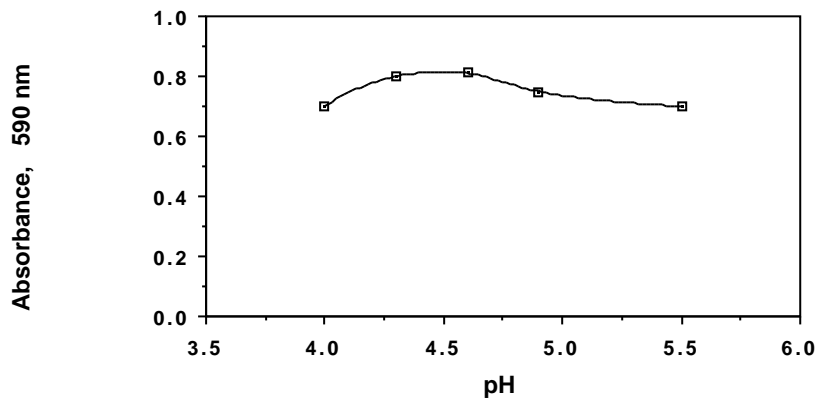


Figure 2. Effect of pH on the activity of *Trichoderma* sp. endo-cellulase on Cellazyme C tablets.

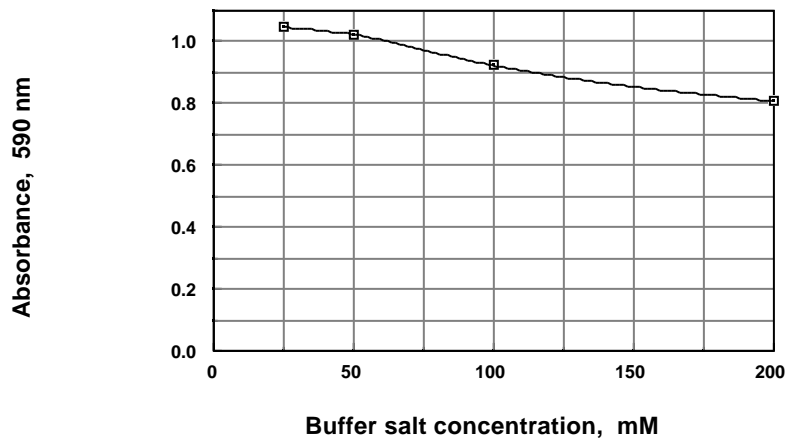


Figure 3. Effect of buffer salt concentration on the activity of *endo*-cellulase on Cellazyme C tablet substrate.



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