Megazyme

ASSAY OF

# *endo*-1,4-ß-Glucanase (Cellulase)

using

# AZO-XYLOGLUCAN

S-AZXG 06/01

## PRINCIPLE:

The assay procedure is specific for the *endo*-1,4-ß-D-glucanase activity present in cellulase preparations. On incubation of dyed xyloglucan with cellulase, the substrate is depolymerised by an *endo*-mechanism to produce low-molecular weight dyed fragments which remain in solution on addition of a precipitant solution to the reaction mixture. High-molecular weight material is removed by centrifugation, and the colour of the supernatant is measured. *endo*-Cellulase in the assay solution is determined by reference to a Standard Curve.

## SUBSTRATE:

The substrate is partially depolymerised and dyed tamarind xyloglucan. The polysaccharide is dyed with Remazolbrilliant Blue to an extent of about one dye molecule per 20 sugar residues.

## DISSOLUTION:

Powdered substrate (2 grams) is added to 160 mL of hot and vigorously stirring water on a hot-plate stirrer. The heat is turned off and stirring is continued until the solution/slurry is homogeneous (about 20 min). Sodium acetate buffer (10 mL, 2 M, pH 4.5) is added. The solution is cooled to room temperature, the pH is adjusted to 4.5, and the volume is adjusted to 200 mL. This solution is stored at 4°C between use. Under these conditions and barring contamination, it is stable for at least 12 months. A few drops of toluene can be added as a preservative.

The substrate solution should be mixed by shaking before use. The solution is viscous, so it should preferably be dispensed with a positive displacement dispenser (eg. Eppendorf Multipette$^{R}$ with a 5.0 mL Combitip).

## BUFFER SOLUTION:

**(Sodium Acetate buffer, 100 mM, pH 4.5)**

Glacial acetic acid (6.0 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. Approximately 50 mL is required. The volume is then adjusted to 1 litre.

## PRECIPITANT SOLUTION:

Industrial methylated spirits (IMS) (99% v/v).

## 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 9.0 mL of **extraction / dilution buffer**. This process of dilution is repeated until an enzyme dilution suitable for assay is obtained.

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 **buffer**, as for the liquid enzyme samples.

## ASSAY PROCEDURE:

Pre-equilibrated enzyme solution (0.5 mL) is added to pre-equilibrated substrate solution (1.0 mL), the mixture stirred on a vortex mixer and incubated at 40°C for 10 min.
The reaction is terminated and high-molecular weight substrate is precipitated by the addition of 2.5 mL of IMS with vigorous stirring for 10 sec on a vortex mixer. The reaction tubes are allowed to equilibrate to room temperature for 10 min and are then stirred again and centrifuged at 3,000 rpm (1,000 g) for 10 min. The supernatant solution is directly poured into a spectrophotometer cuvette and the absorbance of blank and reaction solutions measured at 590 nm. Activity is determined by reference to a Standard Curve.

The blank is prepared by adding IMS to the substrate before addition of the enzyme. Usually, only a single blank is required with each set of determinations.

## STANDARD CURVE:

A typical standard curve is shown below. This curve is for pure *Trichoderma* sp. cellulase from Laminex preparation (Genencor International) diluted in 100 mM sodium acetate buffer (pH 4.5).

## CALCULATION OF ACTIVITY:

***endo*-Cellulase** activity is determined by reference to the standard curve to convert absorbance to milliUnits of activity per assay (i.e. per 0.5 mL) 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. 1.0 g/50 mL or 1.0 mL of enzyme added to 49 mL of buffer).

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

Dilution = further dilution of the **Original Extract**.

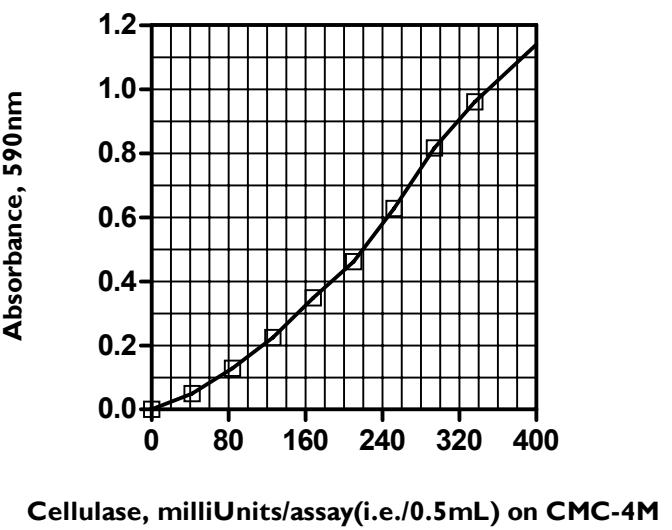


***Figure. 1.*** Trichoderma sp. *endo*-Cellulase standard curve on Azo-Xyloglucan (Lot 10601).

## NOTES:

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