

Megazyme

ASSAY OF
endo-1,4- β -Glucanase
(Cellulase)
using

AZO-CM-CELLULOSE

ACMC 02/2000



PRINCIPLE:

The assay procedure is specific for the *endo*-1,4- β -D-glucanase activity present in cellulase preparations. On incubation of dyed CM-cellulose 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 CM-Cellulose 4M. The polysaccharide is dyed with Remazolbrilliant Blue R to an extent of about one dye molecule per 20 sugar residues.

DISSOLUTION:

Powdered substrate (2 grams) is added to 80 ml of boiling 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 (5 ml, 2M, 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 100 ml. This solution is stored at 4°C between use. Under these conditions and barring contamination, it is stable for at least 12 months.

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 MultipetteR with a 5.0 ml Combipip).

BUFFER SOLUTION:

(Sodium Acetate buffer, 100 mM, pH 4.6)

Glacial acetic acid (6.0 g, 1.05 g/ml) is added to 800 ml of distilled water. This solution is adjusted to pH 4.6 by the addition of 5M (20g/100 ml) sodium hydroxide solution. Approximately 50 ml is required. The volume is then adjusted to 1 litre.

PRECIPITANT SOLUTION:

Dissolve sodium acetate trihydrate (40 grams) and zinc acetate (4 grams) in 150 ml of demineralised water. Adjusted the pH to 5.0 with 5 M hydrochloric acid and the volume to 200 ml with demineralised water. This solution (200 ml) is added to 800 ml of

industrial methylated spirits (IMS, 95%) or ethanol (95 % v/v), mixed well and stored at room temperature in a well sealed bottle.

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 a suitable dilution of the enzyme preparation is achieved. For example, for the industrial enzyme preparation, **Laminex BG** (from *Trichoderma* sp.; Genencor International, U.S.A.) a dilution of the original extract of approximately 100-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 **buffer**, as for the liquid enzyme samples.

ASSAY PROCEDURE:

Pre-equilibrated enzyme solution (0.5 ml) is added to pre-equilibrated substrate solution (0.5 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 Precipitant Solution 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 centrifuged at 3,000 rpm (1,000g) for 10 min. The supernatant solution is directly poured into a spectrophotometer cuvette and the absorbance of blank and reaction solutions measured at 590nm. Activity can be determined by reference to a Standard Curve.

The blank is prepared by adding precipitant solution 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). A suitable dilution for Original Extract of Laminex preparation is approximately 200-fold.

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 0.5ml) on CMC-4M, and then calculated as follows:

Units/ml or gram of Original Preparation:

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

where:

2 = conversion from 0.5 ml to 1.0 ml.

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

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

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

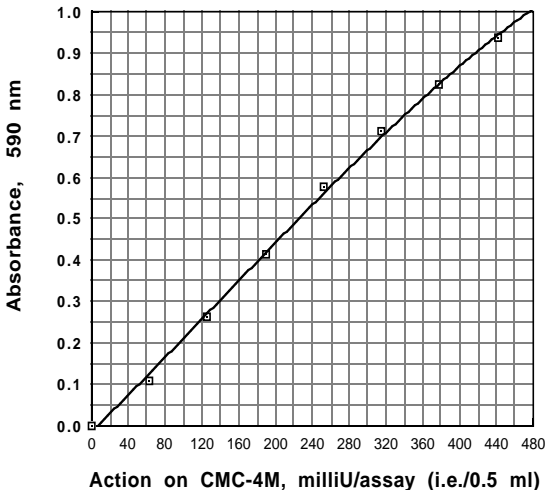


Figure. 1. *Trichoderma* sp. *endo-Cellulase* standard curve on Azo-CM-Cellulose (Lot 60601).



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