

Megazyme

ASSAY OF
endo-1,4- β -Mannanase
using

AZO-CAROB
GALACTOMANNAN

ACGLM 06/00



PRINCIPLE:

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

SUBSTRATE:

The substrate is partially depolymerised and dyed carob galactomannan. The polysaccharide is dyed with Remazolbrilliant Blue R to an extent of about one dye molecule per 30 sugar residues. Powdered substrate (2 grams) is added to 80 ml of hot water (85-90°C) of distilled water and vigorously stirred on a hot-plate stirrer. The heat is turned off and stirring is continued until the substrate completely dissolves (about 10 min). The solution is cooled to room temperature, and sodium acetate buffer (10 ml, 2 M, pH 4.5) is added. The volume is adjusted to 100 ml. This solution is stored at 4°C and is overlaid with a few drops of toluene to prevent microbial contamination. Under these conditions, it is stable for at least 12 months. The substrate solution is viscous, so it should be warmed to room temperature before dispensing, and preferably dispensed with a positive displacement dispenser (eg. Eppendorf MultipetteR with a 5.0 ml Combitip).

ENZYME PREPARATION:

Powdered enzyme preparation (1.0 g) is dispersed in 100 ml of 0.2 M sodium acetate buffer (pH 4.5) and allowed to extract over 15 min. This solution is filtered if necessary, and further diluted in 0.2 M sodium acetate buffer (pH 4.5), as required to obtain the correct range of activity for assay.

Liquid samples (1.0 ml) are added to 99 ml of acetate buffer and further diluted as for the powder samples.

ASSAY PROCEDURE:

Pre-equilibrated enzyme solution (0.5 ml) is added to pre-equilibrated substrate solution (0.5 ml, 2% w/v) and the mixture is stirred on a vortex mixer for 5 sec. 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 ethanol (95% v/v) 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 poured directly from the centrifuge tube into a spectrophotometer cuvette and the absorbance of blank and reaction solutions are measured at 590nm. Activity can be determined by reference to a Standard Curve. The blank is prepared by adding ethanol 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 in Figure 1. This curve is for pure *A. niger* β -mannanase diluted in 0.2 M sodium acetate buffer (pH 4.5) and on substrate Lot 00601. Enzyme activity is standardised using carob galactomannan (0.5%) as substrate in 50 mM sodium acetate buffer (pH 4.5) at 40°C using the Nelson/Somogyi reducing sugar method.

One Unit of activity is defined as the amount of enzyme required to release one micromole of mannose reducing-sugar equivalents per minute under the defined assay conditions.

CALCULATIONS:

Units/ml of original soln

$$= \text{milliUnits per assay (ie. per 0.5 ml)} \times 2 \times \frac{1}{1000} \times \text{Diln.}$$

WHERE:

milliUnits per assay is determined by reference to the Standard Curve

2 = conversion from 0.5 ml to 1.0 ml.

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

Diln. = dilution of the original enzyme solution.

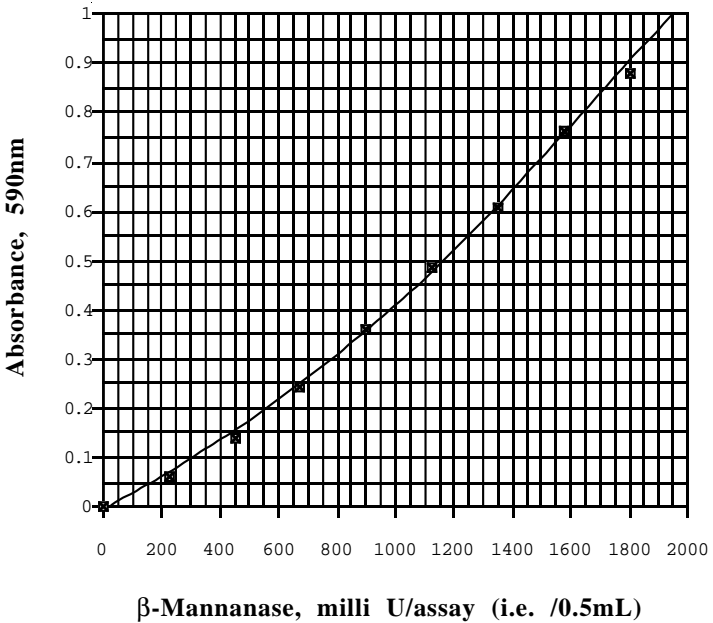


Figure 1. Standard curve for *A. niger* β -mannanase on Azo-Carob Galactomannan (Lot 00601).



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