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ASSAY OF

endo-1,4-β-Mannanase

using

AZO-CAROB GALACTOMANNAN

S-ACGLM 11/18

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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 or a regression equation.

SUBSTRATE:

The substrate is partially depolymerised and dyed carob galactomannan. Carob galactomannan is dyed with Remazol brilliant Blue R to an extent of approx. one dye molecule per 30 sugar residues. To prepare, add 2 g of powdered substrate to 80 mL of hot (85-90°C) distilled water and stir vigorously on a hot-plate stirrer. Turn the heat off and continue stirring until the substrate completely dissolves (about 10 min). Cool the solution to room temperature and add 10 mL of 2 M sodium acetate buffer (pH 4.0). Adjust the volume to 100 mL. Store this solution at 4°C and overlay 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 Multipette® with a 5.0 mL Combitip).

ENZYME PREPARATION:

Add 1 g of powdered enzyme preparation to 100 mL of 0.2 M sodium acetate buffer (pH 4.0) and stir gently over 15 min. If necessary, filter this solution, and dilute further in 0.2 M sodium acetate buffer (pH 4.0) as required (to obtain the correct concentration of activity for assay).

Add 1.0 mL of liquid sample to 99 mL of acetate buffer and dilute further as required, as for the powder samples.

ASSAY PROCEDURE:

Add 0.5 mL of pre-equilibrated enzyme solution to 0.5 mL of pre-equilibrated substrate solution (2% w/v), stir the mixture on a vortex stirrer for 5 sec and incubate at 40°C for 10 min. Terminate the reaction and precipitate high-molecular weight substrate by the addition of 2.5 mL of ethanol (~ 95% v/v) with

vigorous stirring for 10 sec on a vortex mixer. Allow the reaction tubes to equilibrate to room temperature for 10 min and then centrifuge at 3,000 rpm (approx. 1,000 *g*) for 10 min.

Pour the supernatant solution directly from the centrifuge tube into a spectrophotometer cuvette and read the absorbance of the blank and reaction solutions at 590 nm. Determine the activity by reference to a Standard Curve or regression equation.

Prepare the blank by adding ethanol to the substrate before addition of the enzyme preparation. Usually, a single blank only 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 4.0) and on substrate Lot 00603. Enzyme activity was standardised using carob galactomannan (1% w/v) as substrate in 100 mM sodium acetate buffer (pH 4.0) at 40°C using the Nelson/Somogyi reducing sugar method and a D-mannose standard.

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 solution:

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

where:

milli-Units 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 milli-Units to Units

Dilution = dilution of the original enzyme solution

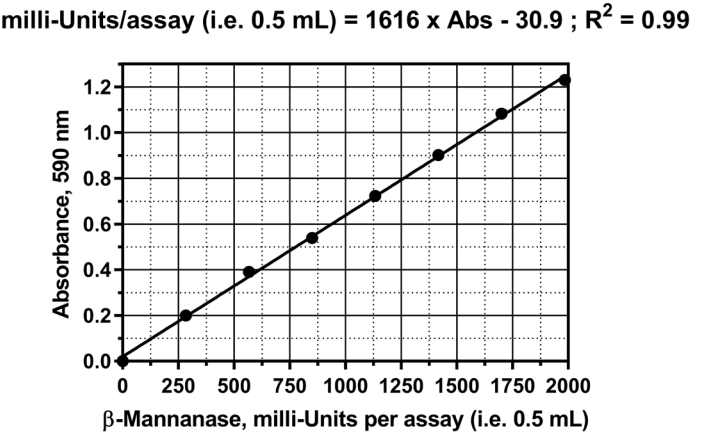

Figure 1. Standard curve for *A. niger* β-mannanase on Azo-Carob Galactomannan (Lot 00603) at pH 4.0.

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