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ASSAY OF

endo-1,4-ß-Galactanase

using

AZO-GALACTAN

S-AGALP 03/14

PRINCIPLE:

This assay procedure is specific for measurement of *endo*-1,4-ß-D-galactanase activity. On incubation of dyed galactan (ex. potato) with *endo*-1,4-ß-D-galactanase, 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*-ß-Galactanase in the assay solution is determined by reference to a Standard Curve.

SUBSTRATE:

The substrate is prepared by incubating potato pectic galactan with arabinofuranosidase to remove most of the arabinose residues. The recovered polysaccharide is then dyed with Remazolbrilliant Blue R to an extent of approx. one dye molecule per 20 sugar residues.

DISSOLUTION OF SUBSTRATE:

Add 2 grams of powdered substrate to 90 mL of boiling and vigorously stirring water. Turn the heat off, and continue stirring the substrate until it completely dissolves (about 10 min). Cool the solution to room temperature, and add 5 mL of 2 M sodium acetate buffer (pH 4.0). Adjust the pH to 4.0 and the volume to 100 mL. Store this solution in a well sealed Duran® bottle at 4°C and overlay with two drops of toluene to prevent microbial contamination. Under these conditions, the substrate is stable for at least 12 months. The substrate solution is viscous, so it should preferably be dispensed with a positive displacement dispenser (e.g. Eppendorf Multipette® with a 5.0 mL Combitip).

PREPARATION OF 95% IMS OR ETHANOL:

Add 960 mL of ethanol (99% v/v) or 960 mL of industrial methylated spirits (IMS, 99% v/v) to a 1 L Duran bottle. Add 40 mL of deionised water and mix well. Keep the bottle well sealed between use.

ASSAY PROCEDURE:

Add 0.5 mL of pre-equilibrated enzyme solution (in 100 mM sodium acetate buffer, pH 4.0 plus BSA, 0.5 mg/mL) to 0.5 mL of pre-equilibrated substrate solution (2% w/v), stir the mixture on a vortex mixer for 5 sec and incubate the tubes at 40°C for 10 min. Add 2.5 mL of ethanol or IMS (95% v/v) with vigorous stirring for 10 sec on a vortex stirrer to terminate the reaction and

precipitate high-molecular weight substrate. Leave the reaction tubes equilibrate at room temperature for 10 min and then stir them again. Centrifuge the tubes at 3,000 rpm (1,800 g) for 10 min. Pour the supernatant solution directly from the centrifuge tube into a spectrophotometer cuvette and read the absorbance of blank and reaction solutions at 590 nm. Activity can be determined by reference to a Standard Curve. The blank is prepared by adding ethanol or IMS (95% v/v) 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-galactanase in 100 mM sodium acetate buffer (pH 4.0) plus BSA (0.5 mg/mL). Enzyme activity is standardised using galactan (potato galactan; 10 mg/mL) as substrate in 100 mM sodium acetate buffer (pH 4.0) at 40°C using the Nelson/Somogyi reducing sugar method and D-galactose as standard.

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

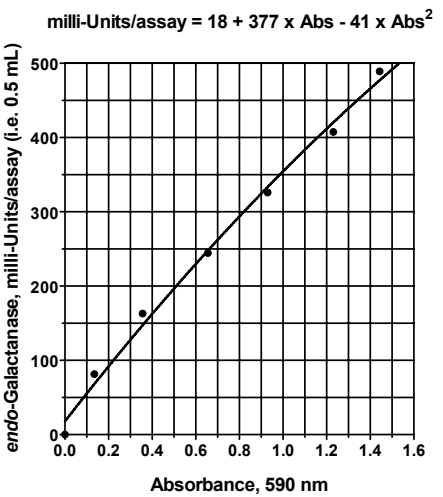

Figure. 1. Standard curve for *Aspergillus* sp. *endo*-Galactanase on Azo-Galactan (Lot 30101)

CALCULATIONS:

Units/mL of original solution

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

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.

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