

# Megazyme

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ASSAY OF  
*endo*-1,4- $\beta$ -Galactanase  
using

**AZO-GALACTAN**

AGALP 11/99



**PRINCIPLE:**

The assay procedure is specific for the *endo*-1,4- $\beta$ -D-galactanase activity. On incubation of dyed galactan with *endo*-1,4- $\beta$ -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*- $\beta$ -Galactanase in the assay solution is determined by reference to a Standard Curve.

**SUBSTRATE:**

The substrate is potato pectic galactan which has been treated with arabinofuranosidase to remove most of the arabinose residues. The polysaccharide is dyed with Remazolbrilliant Blue R to an extent of about one dye molecule per 20 sugar residues. Powdered substrate (2 gram) is added to 90ml of boiling and vigorously stirring water. The heat is turned off, and the substrate is stirred until it completely dissolves (about 10 min). The solution is cooled to room temperature, and 5ml of 2M sodium acetate buffer (pH 4.0) is added. The pH is adjusted to 4.0 and the volume is adjusted to 100ml. This solution is stored at 4°C and is overlain 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 preferably be dispensed with a positive displacement dispenser (eg. Eppendorf Multipipette® with a 5.0ml Combitip).

**ASSAY PROCEDURE:**

Pre-equilibrated enzyme solution (0.5ml) in 0.1M sodium acetate buffer (pH 4.0) is added to pre-equilibrated substrate solution (0.5ml, 2% w/v) and the mixture is stirred on a vortex mixer for 5 sec and incubated at 40°C for 10 minutes. The reaction is terminated and high-molecular weight substrate is precipitated by the addition of 2.5ml of ethanol (95% v/v) with vigorous stirring for 10 seconds on a vortex mixer. The reaction tubes are allowed to equilibrate to room temperature for 10 minutes and are then centrifuged at 3,000 rpm (1,000g) for 10 minutes.

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.

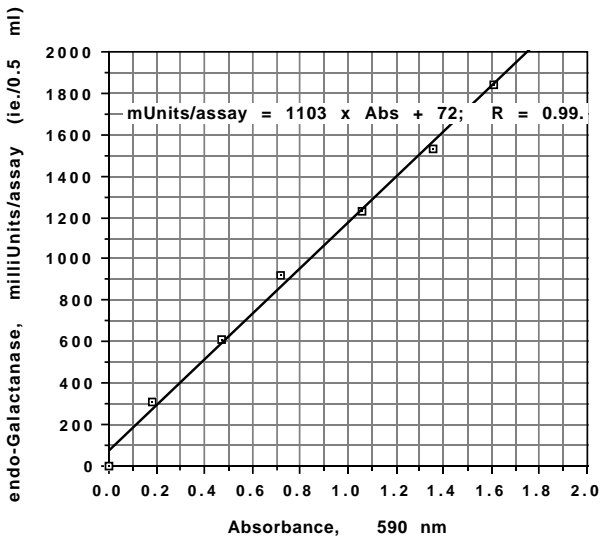
Activity can be determined by reference to a Standard Curve.

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 below. This curve is for pure *A. niger*  $\beta$ -galactanase in 100mM sodium acetate buffer (pH 4.0). Enzyme activity is standardised using galactan (ex. potato; 1% w/v) as substrate in 50mM 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 galactose reducing-sugar equivalents per minute under the defined assay conditions.**



**Figure. 1.** Standard curve for *Aspergillus* sp. *endo*-Galactanase on Azo-Galactan (Lot 50701)

## CALCULATIONS:

Units/ml of original solution

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

**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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