Megazyme

ASSAY OF

RHAMNOGALACTURONANASE

using

AZO-RHAMNOGALACTURONAN

S-AZRH 11/99

SUBSTRATE:

Rhamnogalacturonan is prepared from soybean fibre by exhaustive hydrolysis with a range of pectolytic and cellulosic enzymes and protease. The enzyme preparations are free of enzymes active on rhamnogalacturonan. The neutral sugar profile of this polysaccharide is shown in the gas liquid chromatography trace shown below. Sugars other than rhamnose and galacturonic acid are present, however, these are resistant to cleavage by enzymes such as *endo*-xylanase and α-L-arabinofuranosidase. The purified polysaccharide is dyed to produce soluble azurine rhamnogalacturonan (AZO-Rhamnogalacturonan).

DISSOLUTION:

Powdered substrate (1 gram) is added to 45 mL of warm and vigorously stirring water on a hot-plate stirrer. Stirring is continued until the polysaccharide completely dissolves (about 5 min). Sodium acetate buffer (2 M, pH 4.5, 2.5 mL) is added and the volume is adjusted to 50 mL.

This solution is stored at 4°C between use. Under these conditions and barring enzymic contamination, it is stable for at least 6 months.

ASSAY PROCEDURE:

Pre-equilibrated enzyme solution (0.2 mL) in 100 mM sodium acetate buffer (pH 4.5) is added to pre-equilibrated substrate solution (0.5 mL), and the mixture is stirred on a vortex mixer and incubated at 40°C for 0, 5, and 10 min. The reaction is terminated and high-molecular weight substrate is precipitated by the addition of 2.0 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 5 min and are then centrifuged at 3,000 rpm (1,000 g) for 10 min.

The absorbance of the supernatant solution is measure at 590 nm.

Unfortunately, the unavailability of pure rhamnogalacturonanase precludes the preparation of a standard curve at this point. This enzyme activity is present in Ultra SP^{R} (an enzyme preparation from Novo Nordisk).

NOTES:

Megazyme International Ireland,
Bray Business Park, Bray,
Co. Wicklow,
IRELAND.

Telephone: (353.1) 286 1220
Facsimile: (353.1) 286 1264
Internet: www.megazyme.com
E-Mail: info@megazyme.com