



High DP XYLOGLUCAN OLIGOSACCHARIDE (Lot 120101a)

O-XGHDP

02/19

PREPARATION:

High DP (degree of polymerisation) Xyloglucan oligosaccharides (HDP-XGO) is produced by exhaustive hydrolysis of tamarind seed xyloglucan with *endo*-cellulase followed by β -galactosidase. The reaction mixture is then treated with sodium borohydride to give complete reduction of reducing sugars to sugar alcohols. The high DP material is then recovered by precipitation with 66% ethanol. The approximate MW of this material is 3,000-4,000 daltons.

HDP-XGO is a useful and relatively specific substrate for the enzyme isoprimeverose-producing xyloglucan hydrolase (IP-P-XGH).

Suggested Assay Procedure for IP-P-XGH:

Incubate 0.2 mL of suitably diluted enzyme preparation with 0.5 mL of HDP-XGO (5 mg/mL) in 0.1 M sodium acetate buffer (pH 4.5) at 40°C. Terminate reaction after 3, 6, 9 and 12 min by the addition of 0.5 mL of Nelson-Somogyi reducing sugar Solution D. Prepare blank by adding Nelson-Somogyi solution D to substrate solution before adding enzyme preparation. Develop colour by the modified Nelson-Somogyi procedure. Glucose standards (50 mg) are run concurrently.

HPLC:

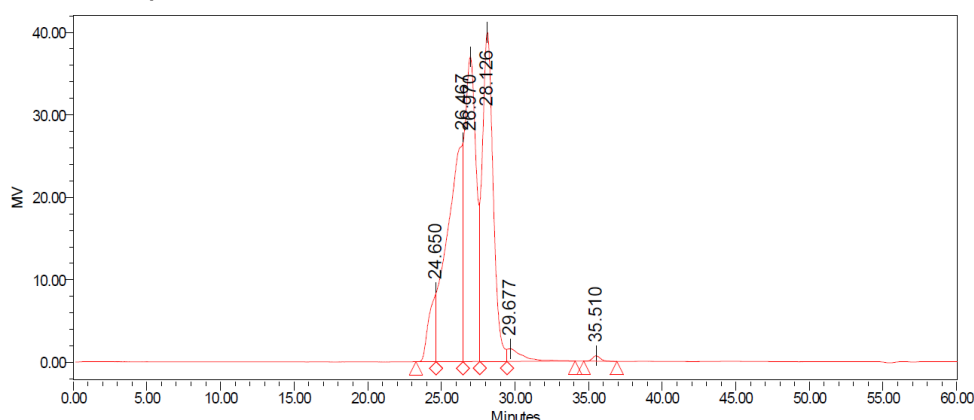
Column: 2 x Tosoh TSK-GEL G2500 PWXL (7.8 x 300 mm) plus guard column (7.8 x 35mm)

Temperature: 80°C

Mobile phase: dH₂O

Flow rate: 0.5 mL/min

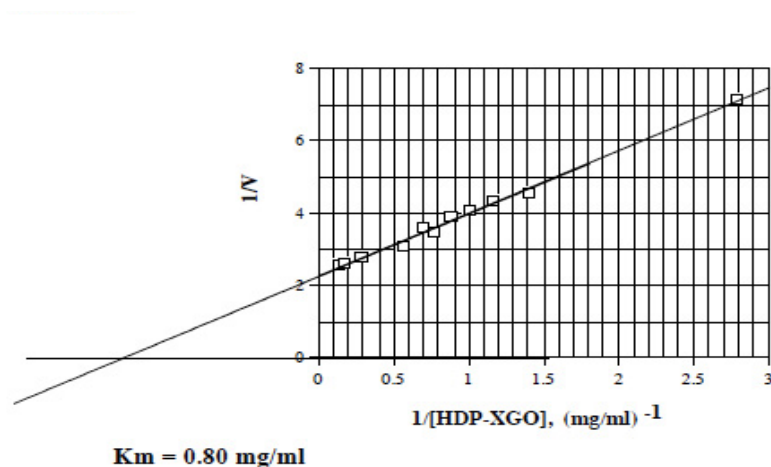
HPLC System: Waters Alliance e2695 Separations Module, Waters 2414 RI detector and Empower v 3 software



Processed Channel: 410

	Processed Channel	Retention Time (min)	Area	% Area	Height
1	410	24.650	281810	4.40	8372
2	410	26.467	1930101	30.11	26527
3	410	26.970	2026344	31.61	36961
4	410	28.126	2038298	31.80	39943
5	410	29.677	110675	1.73	1530
6	410	35.510	23449	0.37	649

Lineweaver- Burke plot of isoprimeverose- producing xyloglucan hydrolase (IP-P-XGH) on HDP-XGO



Reducing Sugars by the Nelson-Somogyi Procedure

NOTE: This method is derived from the published literature. cPoisonous chemicals are used. It is the responsibility of the analyst to ensure that the chemicals are used safely and disposed of properly. Megazyme takes no responsibility for injuries incurred during the correct or incorrect use of this method.

METHOD:

Reagents:

- A.** Dissolve 25 g anhydrous sodium carbonate, 25 g sodium potassium tartrate, and 200 g sodium sulphate in 800 mL of distilled water and diluted to 1 litre. Filter if necessary.
- B.** Dissolve 30 g of copper sulphate pentahydrate in 200 mL of distilled water containing 4 drops concentrated sulphuric acid.
- C.** Dissolve 50 g of ammonium molybdate in 900 mL of distilled water and add 42 mL of concentrated sulphuric acid. Separately, dissolve 6 g of sodium arsenate heptahydrate in 50 mL of water and add this solution to the solution of ammonium molybdate. Adjust the volume to 1 litre. Warm the solution to 55°C if necessary for complete dissolution.
- D.** Add 1 mL of reagent B to 25 mL of reagent A.
- E.** Dilute solution C, 5-fold (50 mL to 250 mL) with distilled water before use (stable at 4°C for about one week).

Reducing Sugars by the Nelson-Somogyi Procedure

Procedure:

To 0.5 L of aqueous sample (containing up to 50 mg of reducing sugar equivalents).

add:

0.5 mL of solution D and mix well on a vortex stirrer.

Heat the tubes in a boiling water bath for 20 min and then cool them to room temperature (5 min). Stir the tubes for 10 sec (until carbon dioxide is completely released).

Add: 3.0 mL of Solution E to each tube. Stir the tubes well (10 sec on vortex mixer), and allow them to stand for 10 min and mix again.

Measure the absorbance at 520 nm.

A blank is prepared by using water (0.5 mL) in place of the sample (0.5 mL).

A standard curve is prepared using appropriate amounts of reducing sugar (e.g. glucose 0-50 mg/test).