Megazyme Building 1,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 2861220 Fax: + 353 1 2861264

Certificate of Analysis

Product: Barley β-Glucan (Medium Viscosity)
Cat. No: P-BGBM
Lot. No: 240849
Expiry Date: N/A

Parameter	Specification	Results
Purity	> 95% (dw basis)	95%
Viscosity	20 – 40 cSt at 1% Solution	30 cSt at 1% Solution*
Starch	< 0.1%	0.04%
Arabinoxylan	< 0.25%	0.10%
Protein	< 0.5%	0.06%
Moisture	< 5%	4.6%
Ash	< 1%	0.20%

*1% w/v solution, 30°C, Ostwald C-type viscometer.

Sugar Composition:
Glucose 94%; (glc of alditol acetates)

This lot meets our specification.

Signature: Kevin O'Leary **Date:** 2024-08-29

Kevin O'Leary

QC Specialist

(*as this certificate is a computer printout, it has not been signed or dated by hand*)

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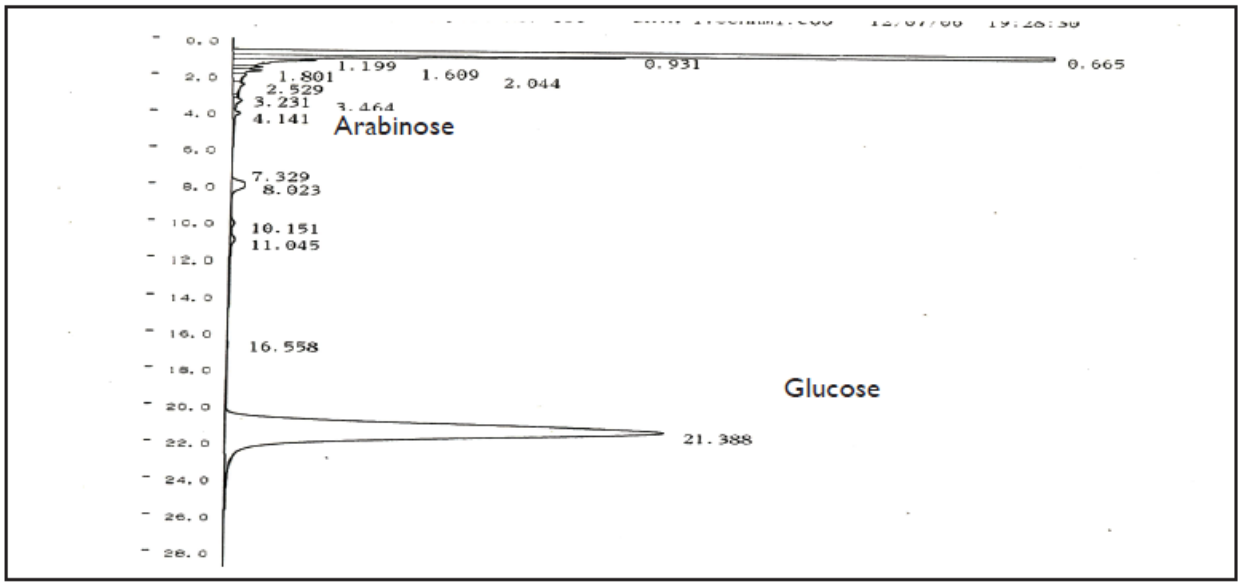
GC:

Gas liquid chromatography of the alditol acetates derived from hydrolysis and derivatization of Barley β-Glucan (Lot 240849)



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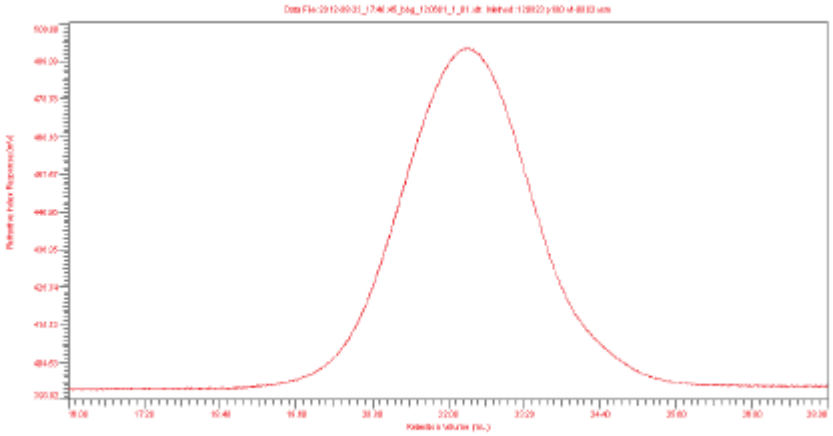
GLC:

A typical polysaccharide sample (~ 10 mg) was hydrolysed using 2N TFA at 120°C for 60 min. Subsequent sodium borohydride reduction was performed in 1N NH4OH for 90 minutes at 40°C. The corresponding alditol acetates were prepared using acetic anhydride and 1-methyl imidazole, extracted into DCM and analysed by GC. Chromatography was performed on a Shimadzu GC-14B with CHROMATOPACK C-R8A using a Packed glass column (6ft x 5 mm OD, 3mm ID) with 3% Silar 10C on W-HP (80-100 mesh). The carrier gas was nitrogen at 130 KPa. Injector temperature; 250°C; Column temperature; 230°C. Detection by FID with 60KPa H2 pressure and 50 KPa air pressure.



Polysaccharide & Lot Number	Mp	Mw	Mn	(η)	Rg	Pd
Barley β-Glucan Lot 240849	196000	265000	141000	3.59	30.0	1.89
Sd	1300	6300	3700	0.01	1.2	0.08

Viscotek Analysis:

Polysaccharide Solubilisation Protocol A few mg (2-6) of the polysaccharide were weighed into a glass test tube in duplicate. Sufficent 0.1 M sodium nitrate containing 5 mM sodium azide (SEC eluent) was added to give a polysaccharide concentration of ~ 1 mg/mL. The samples were stirred for 2.5 hr at 90°C. After cooling to RT, the solutions were filtered through a 0.45 µm filter into auto-sampler vials. This protocol was repeated on two separate days.

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Size Exclusion Chromatography:

The chromatographic system was a Shimadzu SCL-10Avp control unit (Shimadzu Scienti c Instruments, Inc., Columbia, MD) using Shodex OHpak Kb-806M HQ column (Showa Denko K.K., Tokyo, Japan) followed by an Ultrahydrogel linear column (Waters, Milford, CT) maintained at 40°C and run at a flow rate of 0.6 mL/min with an eluent of 100 mM NaNO3 containing 5 mM NaN3. All measurements were made from data collected using a model 305 Triple Detector Array (TDA) from Viscotek (Viscotek, Houston, TX), which consisted of a refractive index detector, a differential pressure detector, a right angle laser light scattering detector (RALS) and a low angle laser light scattering detector (LALS). Values were calculated using OmniSEC 4.6 software (Viscotek, Houston, TX). A refractive index increment of 0.146 mL/g was used for the calculations. Pullulan standards were used to calibrate the method. For each run, two standards were used. The p100 standard was used to calibrate method, while the p800 was treated as a sample in order to confirm the accuracy of the method. Both standards were from Fluka and were prepared in eluent.

Results:

The results of the analyses are provided in the table below. The average of three determinations is reported with the standard deviation (sd).

The parameters measured are:

Mp – peak molecular weight (g/mol) – the molecular weight of the most abundant species in the sample.

Mw – weight average molecular weight – the average molecular weight of the distribution based on the weight of particles in each fraction.

Mn – number average molecular weight – the average molecular weight of the distribution based on number of particles in each fraction.

[η] – intrinsic viscosity (dL/g) – the contribution of solute molecules to solution viscosity.

Rg – radius of gyration (nm) – the root mean square distance of the monomers from the centre of the molecule.

Pd – Polydispersity Index – the ratio of Mw/Mn which is generally used as an indicator of the width of the distribution, with 1.0 representing monodisperse molecules.

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