β-GLUCAN MW STANDARDS

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Reported molecular weight values for high molecular weight oat and barley β-glucan range from several hundred thousand to 10 million daltons. This variation could be due to either differences in the extraction procedures resulting in varying degrees of depolymerisation, or alternatively, to differences in the analytical procedures used to characterise the purified polymers.

To help resolve some of these problems, Megazyme offers a range of β-glucan molecular weight standards. The method of polysaccharide dissolution and MW determination is described below.

Sample Solubilization Protocol:

A few mg (2-6) of each sample was weighed into a glass test tube in duplicate. Sufficient 0.1 M sodium nitrate containing 5 mM sodium azide (SEC eluent) was added to a concentration of ~1 mg/ml. Samples were stirred 2.5 hours at 90°C. After cooling to RT, samples were filtered through a 0.45mm filter into auto-sampler vials. This protocol was repeated on two separate days.

Size Exclusion Chromatography:

The chromatographic system was a Shimadzu SCL-10Avp control unit (Shimadzu Scientific 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 $NaNO_3$ containing 5 mM NaN_3. 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.

PROPERTIES OF β-GLUCANS:

Starch: < 0.1%   Moisture: ~ 5%
Protein: < 0.2%   Appearance: white powder.

STORAGE OF POWDERS:

Store dry at room temperature in well sealed containers. Under these conditions, the product is stable for > 20 years

Barley and Oat β-Glucan Molecular Weight Standards

Sample name & Lot number	Mp	Mw	Mn	[η]	Rg	Pd
A. Barley BG – Lot 10404a	667000	650000	481000	6.13	54.1	1.35
sd	17000	12000	10800	0.19	1.7	0.03
B. Oat BG – Lot 90801a	375000	391000	307000	4.10	39.5	1.27
sd	10600	9100	6800	0.15	3.9	0.01
C. Oat BG – Lot 71102a	247000	265000	192000	3.11	29.0	1.38
sd	3830	3260	2120	0.02	2.67	0.02
D. Barley BG – Lot 90801a	160000	229000	187000	3.05	35.14	1.44
sd	5440	4810	2630	0.03	2.27	0.02
E. Oat BG – Lot 110303a	67100	70600	56000	1.32	nc	1.26
sd	3090	2680	2790	0.06		0.02
F. Oat BG – Lot 110301a	33600	35600	30200	0.84	nc	1.18
sd	4610	10400	3940	0.03	3.22	0.10

In the table above, each value represents the average of three determinations, with the standard deviation (sd).

The parameters measured were:

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 of 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.

Size Exclusion Chromatography of β-glucan samples