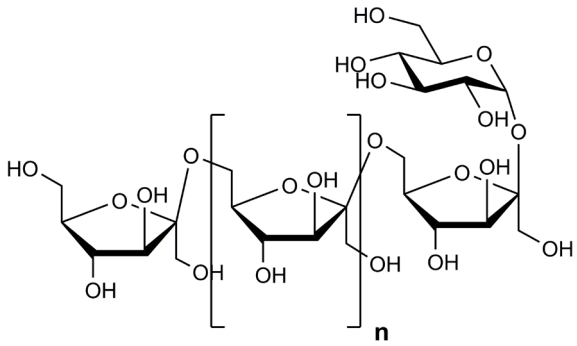
LEVAN (Lot 171114)

CAT. NO: **P-LEVAN** **04/18**
CAS: **9013-95-0**



Schematic representation of levan

DESCRIPTION

Levan is a linear fructan polymer composed of β-2,6 linked fructofuranose residues. Most of the fructan chains terminate with a β-2,1 linked glucopyranose residue.

PREPARATION

Levan is prepared by extraction from Timothy grass and purified.

PROPERTIES

Purity (DWB):	> 98%
Molecular Weight:	~ 12.5 kDa
Sugar Composition (by NMR):	Fructose:Glucose = 98.7:1.3
Protein:	< 0.2%
Ash:	1.0%
Moisture:	4.5%
Physical Description:	Off-white powder
Solubility:	> 1.0% (w/v)

STORAGE CONDITIONS

Store dry at room temperature in a well-sealed container. Under these conditions, the product is stable for > 10 years.

SIZE-EXCLUSION CHROMATOGRAPHY (SEC)

System: Agilent Technologies 1260 Infinity II LC system

Column: Superose 12 GE-Amersham, optimal separation range 1,000-180,000 Da for dextrans, equilibrated with 50 mM ammonium acetate, pH 5.5 buffer.

Detection: RID detector (Agilent) was used for post-column detection of non-volatile, soluble components eluted from the column. Gain 8, filter 5, 70°C

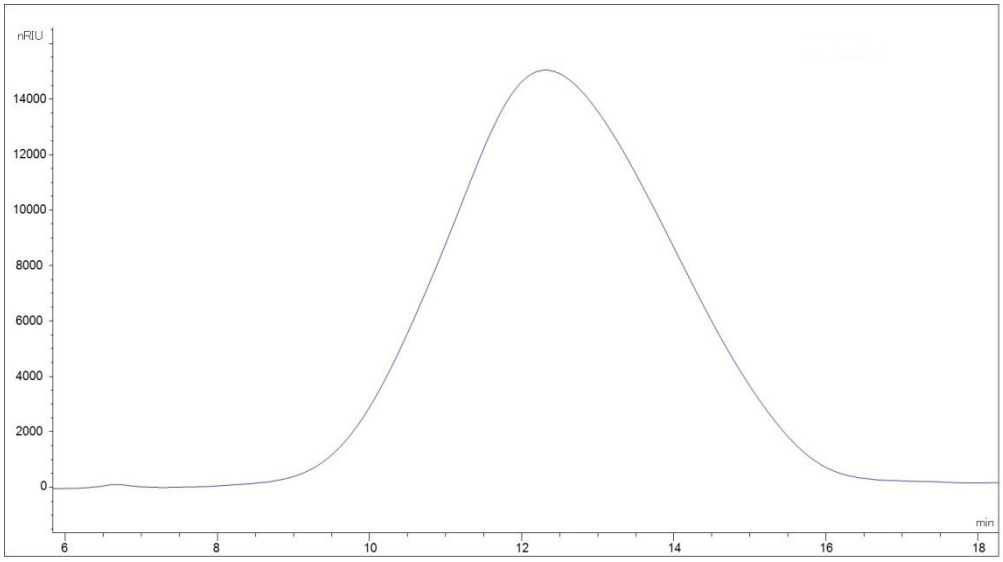
Flow rate: 1.0 mL/min

Sample: 4 mg/mL solution in 50 mM NH_4Ac-buffer; 400 µg was injected

Before injection to the SEC column, insoluble parts of the sample were removed by filtration through 0.45 micron filter.

Elution buffer: 50 mM ammonium acetate, pH 5.5.

Data were collected and processed by Agilent OpenLAB ChemStation software.



P-LEVAN	Mp	Mw	Mn	Pd
Lot 171114	~ 10,000	~ 12,500	~ 6,500	1.9

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

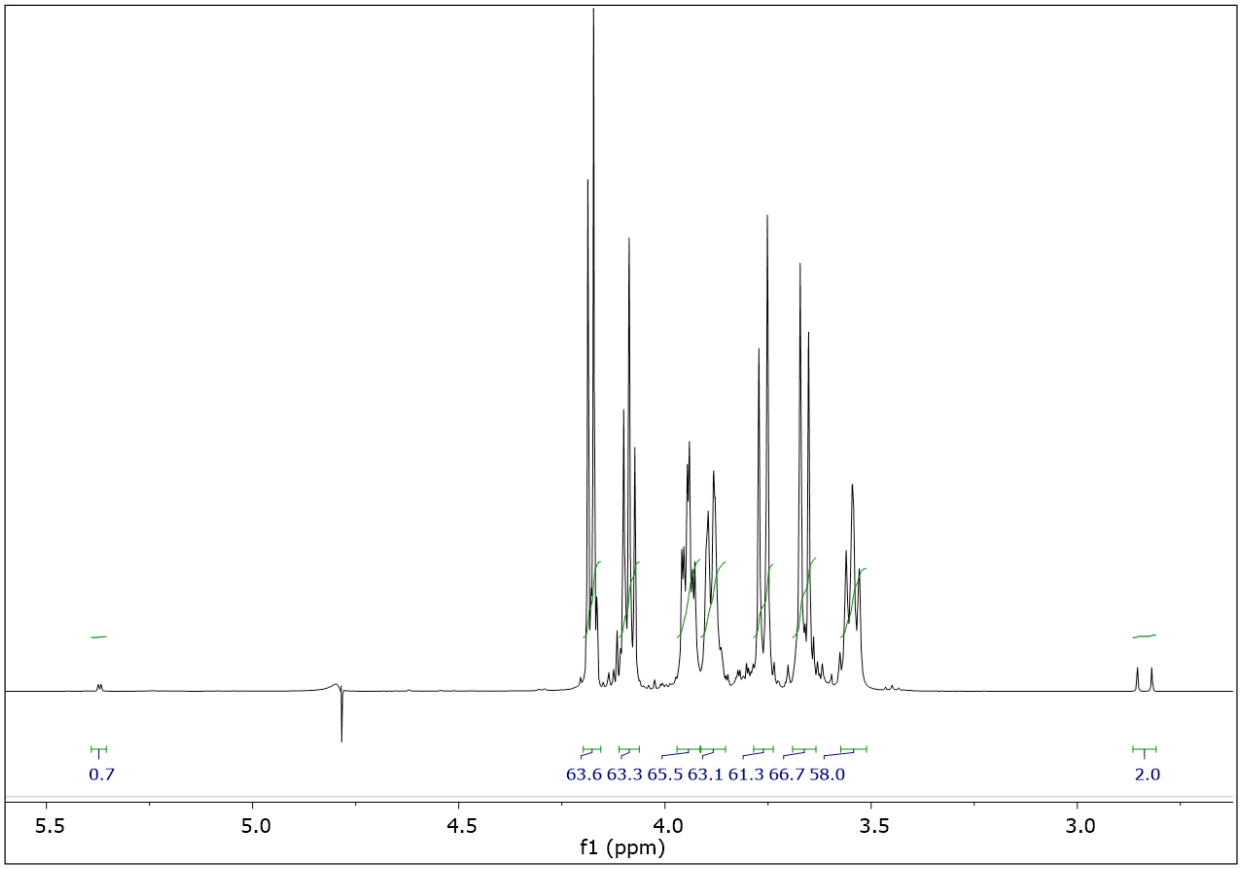
Mw – weight average molecular weight – the average molecular weight of the distribution based on the weight of particles in each fraction.

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

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.

NMR ANALYSIS

The sample (15 mg) was deuterium-exchanged by lyophilisation in D_2O (99.9% D, Sigma). The sample was re-dissolved in 0.5 mL D_2O (Cambridge Isotope Laboratories, 99.96% D). The solution was transferred into a 5-mm OD NMR tube. 1-D Proton spectra were acquired on a Varian Inova-600 MHz spectrometer at 25°C. Chemical shifts were measured relative to internal acetone (δH=2.22 ppm). The glucose:fructose ratio of 98.7:1.3 was obtained here by integration of the relevant signals. Note that the trace below includes an internal standard, phosphonoacetic acid (δH=2.82, 2.85 ppm, 88ug).



No	Chemical shift, δ ppm	Multiplicity, J1,2 Hz	Assignment	Integral values
Ref	2.85, 2.82	2 singlets	PAA	2.0
1	3.58-3.51	multiplet, 8Hz	β-Frcf H-6a	58.0
2	3.66	doublet, 12.3Hz	β-Frcf H-1a	66.7
3	3.76	doublet, 12.3Hz	β-Frcf H-1b	61.3
4	3.90-3.87	multiplet	β-Frcf H-6b	63.1
5	3.94	doublet of triplets	β-Frcf H-5	65.5
6	4.09	triplet, 8.0Hz	β-Frcf H-4	63.3
7	4.18	doublet, 8.3Hz	β-Frcf H-3	63.6
8	5.37	doublet, 3.8Hz	α-Glcp	0.71

These results coincide with previously reported bacterial levans (Shih & Yu, J. (2005). *Agri. Food Chem*., 53, 8211-8215) and indicate a polysaccharide of the levan type with a β-(2-6)-fructofuranosyl linkage.

HPAEC-PAD ANALYSIS

A stepwise linear gradient method was employed on a Dionex ICS5000 + DP equipped with Dionex CarboPac PA200 guard and analytical columns (3 x 250 mm) as shown.
Detector: Au electrode; waveform Carbohydrate, standard quad
Flow rate: 0.5 mL/min
Temperature: 30°C

Gradient (min)	100 mM NaOH/ 120 mM NaOAc (%)	100 mM NaOH/ 320 mM NaOAc (%)
0	100	0
40	0	100
50	0	100
50.5	100	0
60	100	0