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

Certificate of Analysis

Product: Levan
Cat. No: P-LEVEN
Lot. No: 180620A
Expiry Date: N/A

Parameter	Specification	Results
Purity	> 98% (dw basis)	98.7 %
Protein	< 1%	0.7 %
Moisture	< 5%	1 %
Ash	< 2%	0.5 %

Sugar Composition (by NMR):
Fructose : Glucose = 98.7 : 1.3

Solubility:
> 1.0% (w/v)

This lot meets our specification.

Signature: 

Date: 05.Jun.2020

Elaine Delaney
Quality Control Specialist

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

© 2023, Neogen Corporation; © 2023, Megazyme. All rights reserved.
Neogen is a registered trademark of Neogen Corporation. Megazyme is a registered trademark of Megazyme Ltd.

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

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

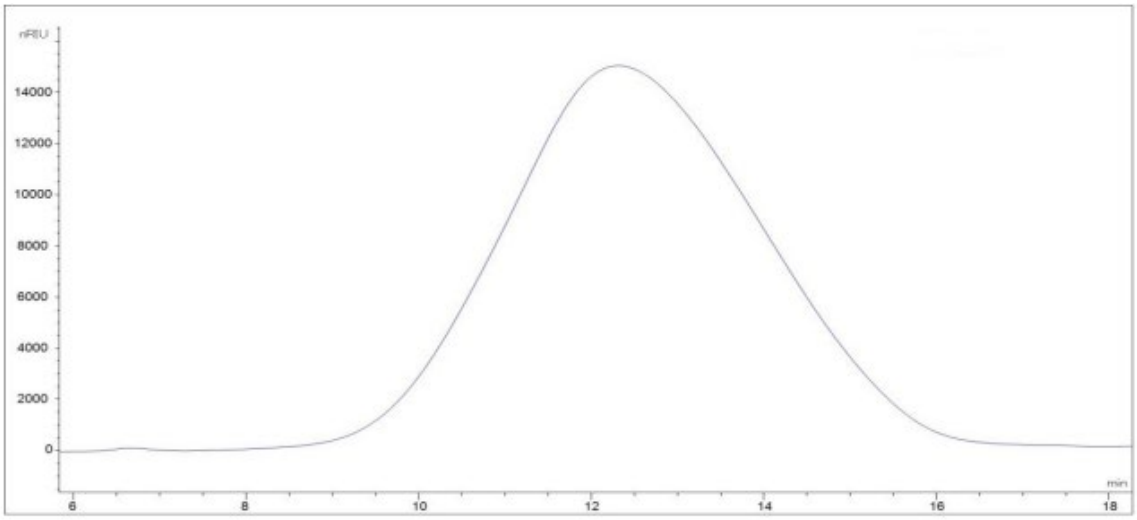
Flow rate: 1.0 mL/min

Sample: 4 mg/mL solution in 50 mM NH4 Ac-buffer; 400 µg was injected

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

Elution buffer: 50 mM ammonium acetate, pH 5.5.

Data was collected and processed by Agilent OpenLAB ChemStation software.



P-LEVAN	Mp	Mw	Mn	Pd
	~ 10,000	~ 12,500	~ 6,500	51.9

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.

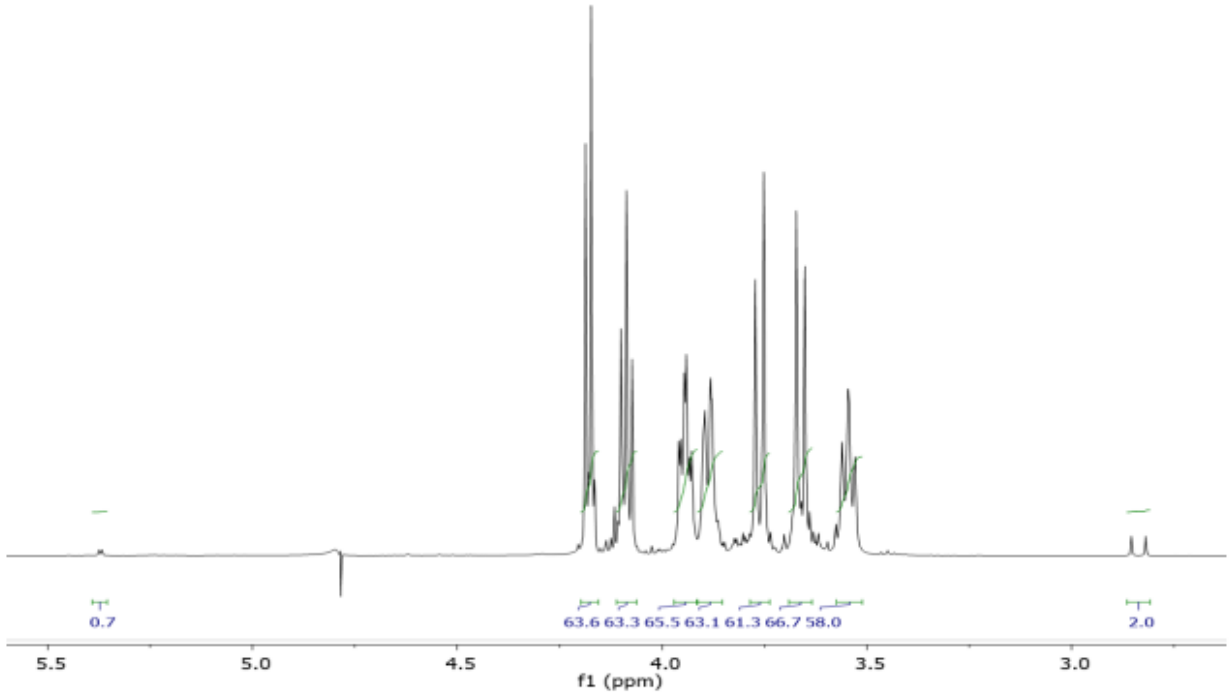
© 2023, Neogen Corporation; © 2023, Megazyme. All rights reserved.
Neogen is a registered trademark of Neogen Corporation. Megazyme is a registered trademark of Megazyme Ltd.

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

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 D2 O (99.9% D, Sigma). The sample was re-dissolved in 0.5 mL D2 O (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 (dH=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 (dH=2.82, 2.85 ppm, 88ug).



© 2023, Neogen Corporation; © 2023, Megazyme. All rights reserved.
Neogen is a registered trademark of Neogen Corporation. Megazyme is a registered trademark of Megazyme Ltd.

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

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.

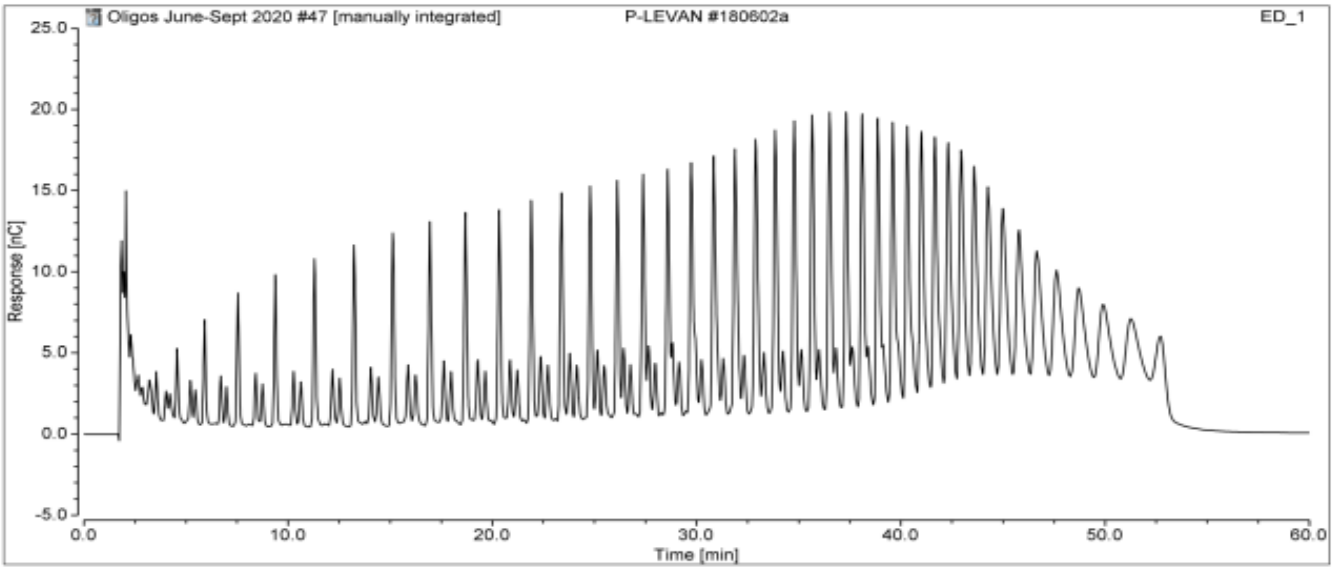
© 2023, Neogen Corporation; © 2023, Megazyme. All rights reserved.
Neogen is a registered trademark of Neogen Corporation. Megazyme is a registered trademark of Megazyme Ltd.

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

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



© 2023, Neogen Corporation; © 2023, Megazyme. All rights reserved.
Neogen is a registered trademark of Neogen Corporation. Megazyme is a registered trademark of Megazyme Ltd.