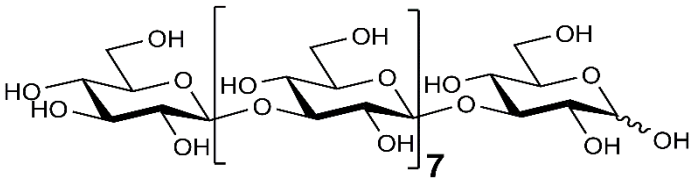
LAMINARINONAOSE (Lot 190909)

O-LAM9 **09/19**

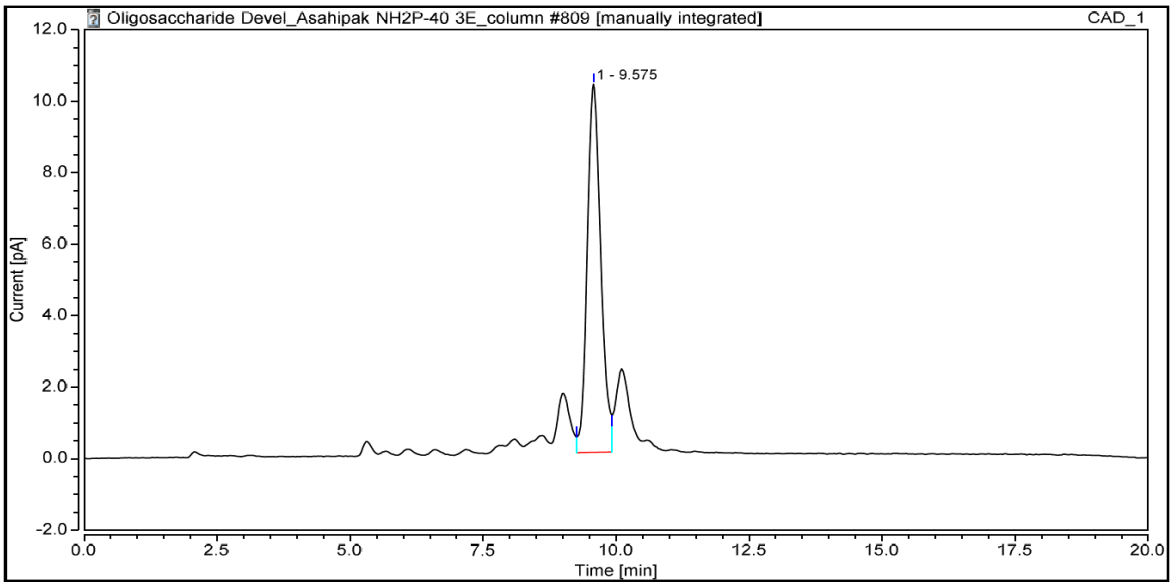
CAS: **112916-37-7**
Molecular Formula: $C_{54}H_{92}O_{46}$
MW: **1477.3**



PURITY: > 65% (HPLC)

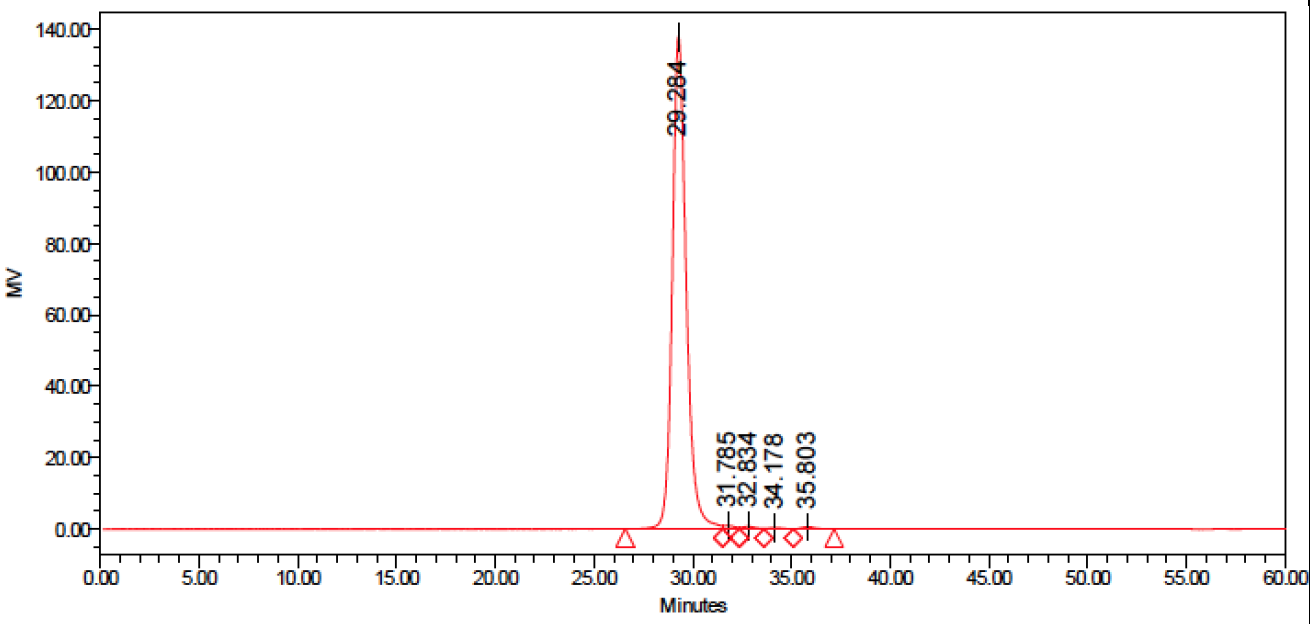
HPLC:
Column: Shodex Asahipak NH2P-40 3E analytical column
Temperature: 35°C
Flow rate: 0.35 mL/min (Eluent gradient shown below)
Detector: CAD (Charged Aerosol Detector)
HPLC System: Thermofisher U3000 Ultimate and Chromeleon v 7.0 software

Time (min)	H_2O (%)	CH_3CN (%)
0	40	60
1	40	60
14	60	40
16	40	60
20	40	60



HPLC:

Column: 2 x Tosoh TSK-GEL G2500 PWXL (7.8 x 300 mm) plus guard column (7.8 x 35 mm)
Temperature: 80°C
Mobile phase: dH_2O
Flow rate: 0.5 mL/min
HPLC System: Waters Alliance e2695 Separations Module, Waters 2414 RI detector and Empower v 3 software



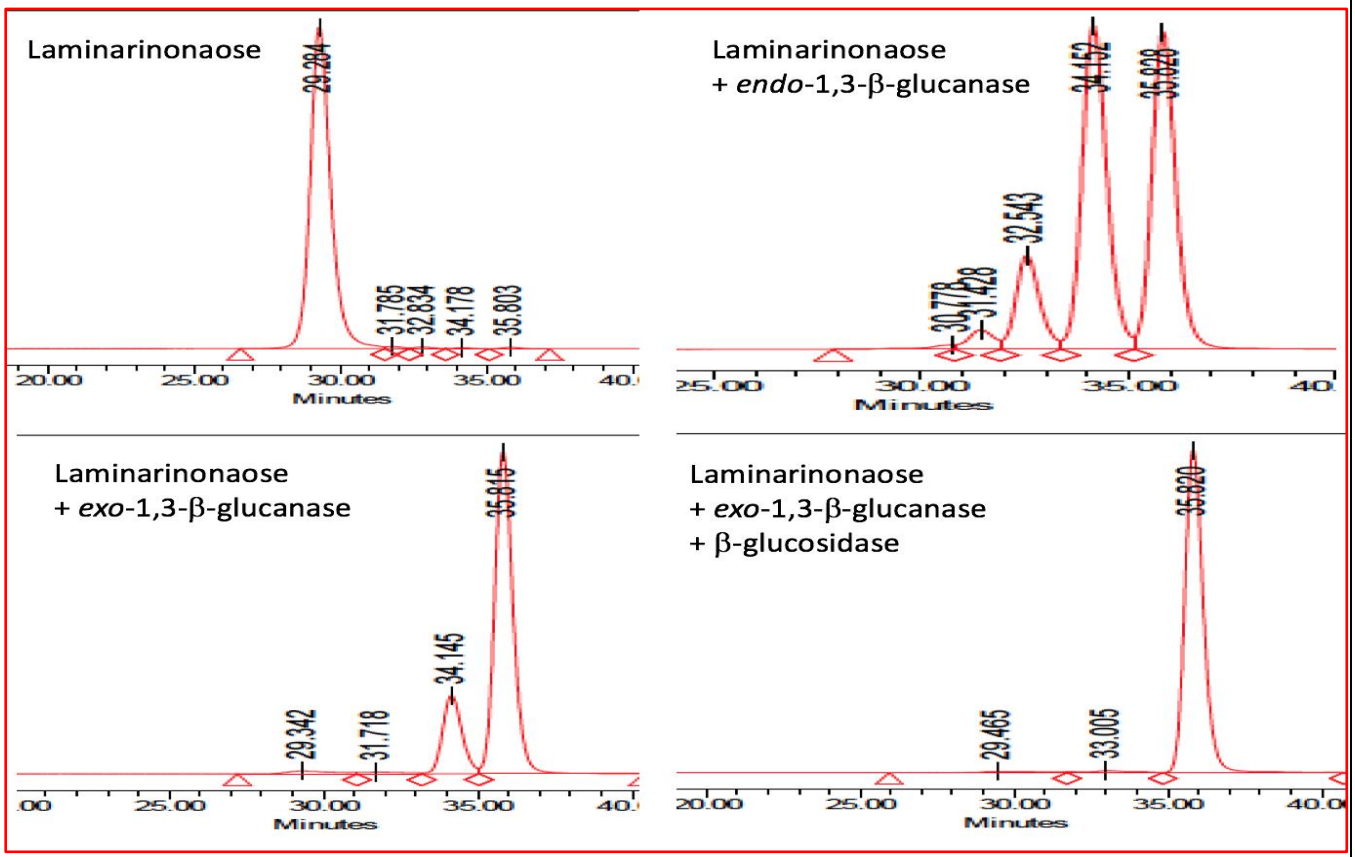
Hydrolysis of laminarinonaose by *endo*-1,3-β-glucanase, *exo*-1,3-β-glucanase and β-glucosidase

Purity of laminarinonaose was studied by hydrolysis by *endo*-1,3-β-glucanase, *exo*-1,3-β-glucanase and β-glucosidase. Incubations were performed as follows:

1. Hydrolysis by *endo*-1,3-β-glucanase – 1 mL of laminarinonaose (10 mg/mL) in 10 mM sodium acetate buffer (pH 4.5) was incubated with 0.2 mL of *endo*-1,3-β-glucanase (10 U) (Megazyme cat. No. **E-LAMSE**) at 40°C for 1 h. Reaction was terminated by heating the solution at 100°C for 5 min and the solution was centrifuged at 13,000 rpm for 5 min. Samples were analysed by HPLC on 2 columns of Tosoh TSK-GEL G2500 PWXL (7.8 x 300 mm) plus guard column (7.8 x 35 mm). Temperature: 80°C. Samples were deionized inline with cation and anion exchange guard cartridges, H^+ and CO_2^{3-} forms respectively (Bio-Rad Laboratories, Cat. No. 125-0118).

2. Hydrolysis by *exo*-1,3-β-glucanase – 1 mL of laminarinonaose (10 mg/mL) in 10 mM sodium acetate buffer (pH 4.5) was incubated with 0.2 mL of *exo*-1,3-β-glucanase (10 U) (Megazyme cat. No. **E-EXBGTV**) at 40ºC for 1 h. Reaction was terminated by heating the solution at 100°C for 5 min and the solution was centrifuged at 13,000 rpm for 5 min. Samples were analysed by HPLC as for example 1.

3. Hydrolysis by *exo*-1,3-β-glucanase plus β-glucosidase – 1 mL of laminarinonaose (10 mg/mL) in 10 mM sodium acetate buffer (pH 4.5) was incubated with 0.2 mL of *endo*-1,3-β-glucanase (10 U) (Megazyme cat. No. **E-EXBGTV**) plus β-glucosidase (8 U) (Megazyme cat. no. **E-BGLUC**) at 40°C for 1 h. Reaction was terminated by heating the solution at 100°C for 5 min and the solution was centrifuged at 13,000 rpm for 5 min. Samples were analysed by HPLC as for example 1.



Clearly, *endo*-1,3-β-glucanase produces mainly mon- to tri-β-gluco-oligosaccharides, *exo*-1,3-β-glucanase produces glucose and laminaribiose and a mixture of *exo*-1,3-β-glucanase and β-glucosidase gives near complete hydrolysis to glucose. All results are consistent with what would be expected on hydrolysis of a linear laminarioligosaccharide.