

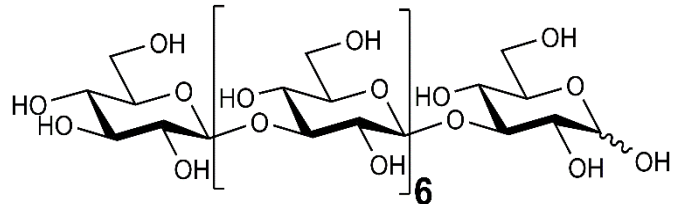


LAMINARIOCTAOSE (Lot 190908)

O-LAM8

09/19

CAS: 72621-54-6
Molecular Formula: C₄₈H₈₂O₄₁
MW: 1315.1



PURITY: > 75% (HPLC)

HPLC:

Column: Shodex Asahipak NH2P-40 3E analytical column

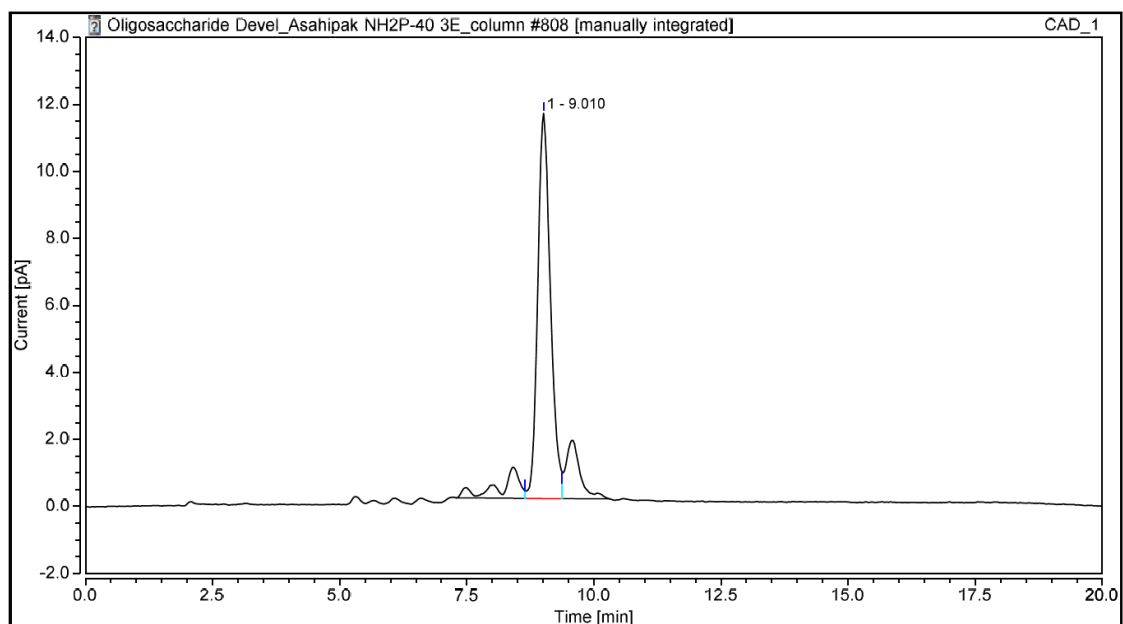
Temperature: 35°C

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 ₂ O (%)	CH ₃ CN (%)
0	40	60
1	40	60
14	60	40
16	40	60
20	40	60



HPLC:

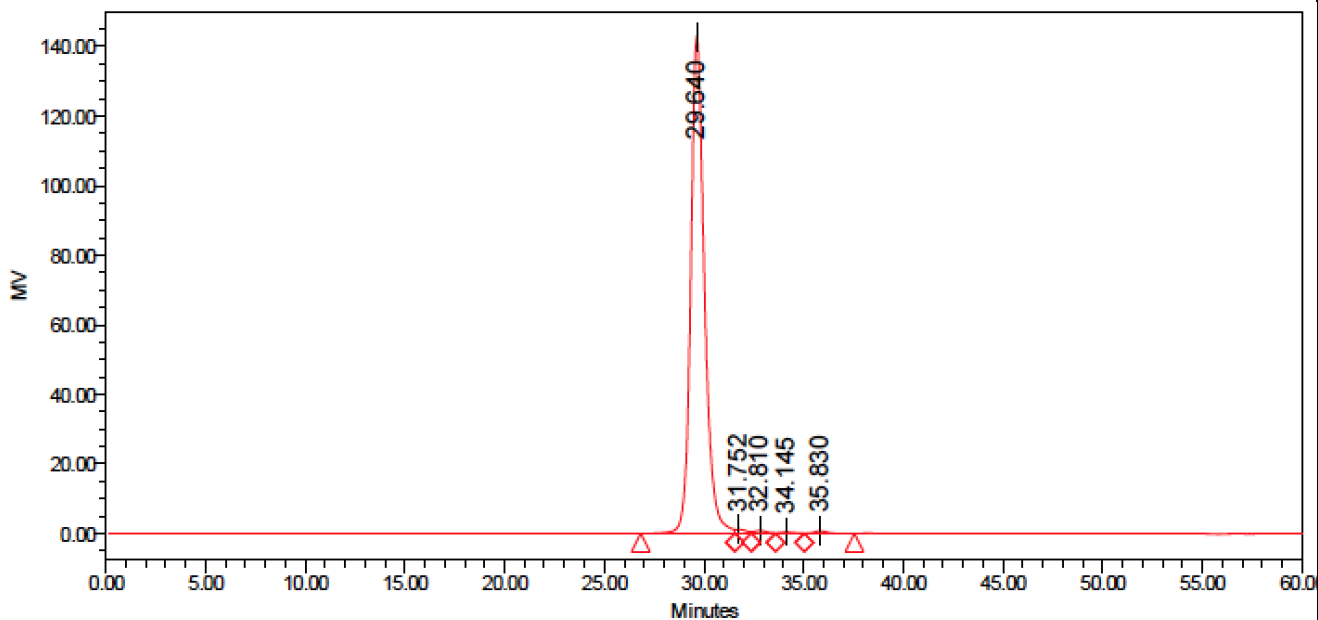
Column: 2 x Tosoh TSK-GEL G2500 PWXL (7.8 x 300 mm) plus guard column (7.8 x 35 mm)

Temperature: 80°C

Mobile phase: dH₂O

Flow rate: 0.5 mL/min

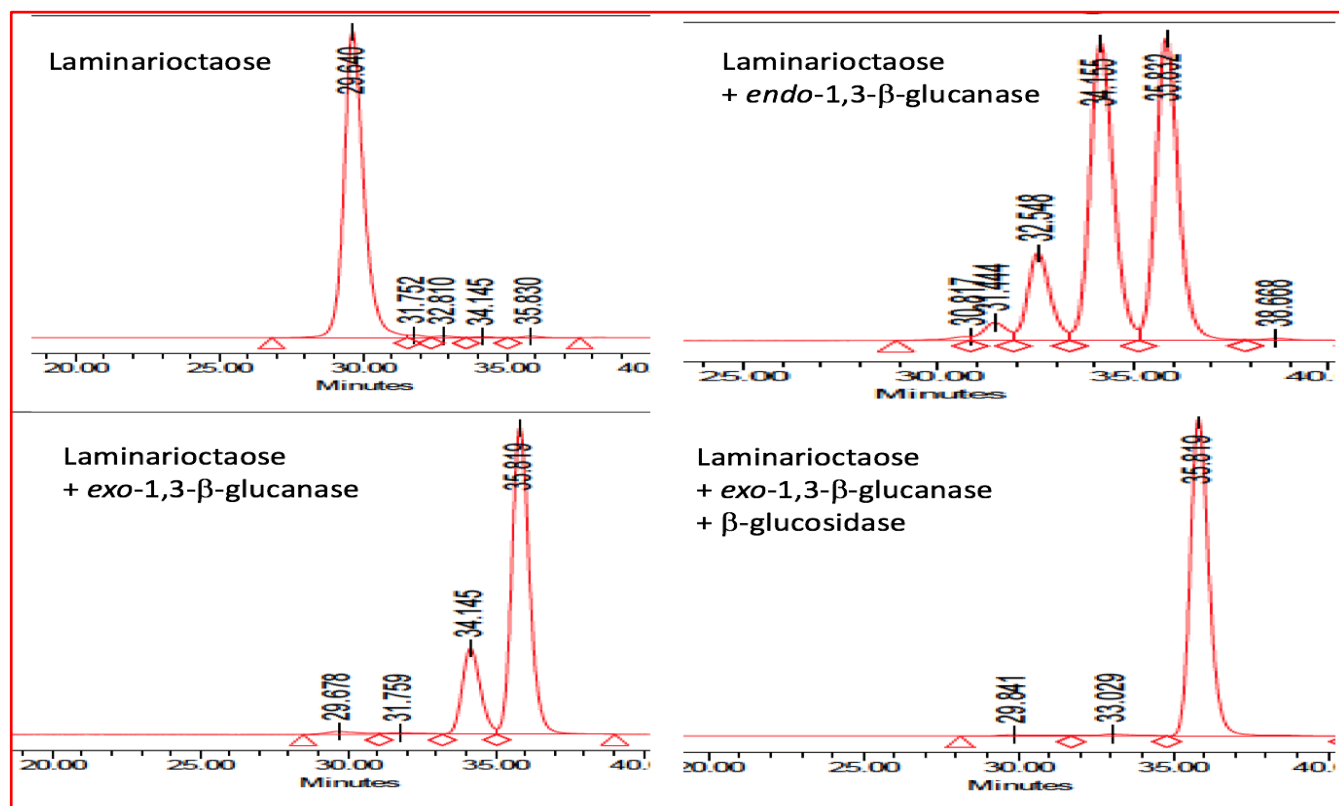
HPLC System: Waters Alliance e2695 Separations Module, Waters 2414 RI detector and Empower v 3 software



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

Purity of laminarioctaose 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 laminarioctaose (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 CO₂³⁻ forms respectively (Bio-Rad Laboratories, Cat. No. 125-0118).
2. Hydrolysis by *exo*-1,3- β -glucanase – 1 mL of laminarioctaose (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 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.
3. Hydrolysis by *exo*-1,3- β -glucanase plus β -glucosidase – 1 mL of laminarioctaose (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-**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.