PRODUCT SPECIFICATION 73484

Dionex CarboPac PA300-4µm column

High-efficiency and high-resolution glycan analysis

Keywords: Pulsed amperometry, derivatization-free, direct detection, gold electrodes, oligosaccharide, glycome, glycomics, mucin, HPAE-MS, glycosylation, carbohydrate, ion chromatography, HPAE-PAD

Benefits

- Total glycome analysis with isomer resolution
- Resolution of neutral and charged glycans
- Free and released glycan analysis
- Oligosaccharide analysis for biologics and food samples
- Simple, direct approach using pulsed amperometric detection
- No derivatization required
- Compatible with mass spectrometry for HPAE-MS applications

The Thermo Scientific™ Dionex™ CarboPac™ PA300-4µm column is an anion-exchange column primarily designed for the analysis of complex oligosaccharides from biologics and food samples. This column is complementary to the Thermo Scientific™ Dionex™ CarboPac™ PA200 column for high-efficiency and high-resolution separations of neutral glycans. For applications requiring a range of glycan separations with varying size and charge states, the Dionex CarboPac PA300 column is the recommended column with which to begin analysis. This column is designed for efficient separations of complex, heterogeneous samples with good chromatographic resolution of neutral and charged glycans of different sizes.

High-efficiency and high-resolution separations of oligosaccharides

There is a significant and increasing demand for reproducible, fast, and simple methods for the profiling of oligosaccharides in biologicals and in the food industry. Although a variety of HPLC approaches are proposed for these applications, in general, most prove inadequate for separating complex oligosacharide mixtures and are limited by their nonspecific nature and low limits of detection.

The Dionex CarboPac PA300 column provides high-resolution separations of complex oligosaccharides using a technique known as high-performance anion-exchange (HPAE) chromatography with pulsed amperometric detection (PAD). HPAE-PAD allows for separations at high pH and detection without the need for derivatization.

The major advantages of HPAE-PAD are (1) fast analyses, (2) ease of use—samples are directly analyzed without the need for derivatization and little to no sample cleanup, (3) low- to sub-picomole range sensitivity, and (4) high resolution (e.g., separation of anomeric and positional isomers—HPAE separates all classes of oligosaccharides according to structural features such as size, charge, composition, anomericity, and linkage isomerism).

The Dionex CarboPac PA300-4µm column packing material has a unique structure composed of a highly crosslinked core and a nanobead anion-exchange layer attached to the surface as illustrated in Figure 1. The Dionex CarboPac PA300-4µm substrate is composed of supermacroporous, 4µm diameter resin beads consisting of ethylvinylbenzene crosslinked with 55% divinylbenzene. The anion-exchange layer is functionalized with quaternary ammonium functional groups. The latex nanobead anion-exchange layer has a controlled thickness, which results in excellent mass transfer characteristics and, consequently, very high efficiency.

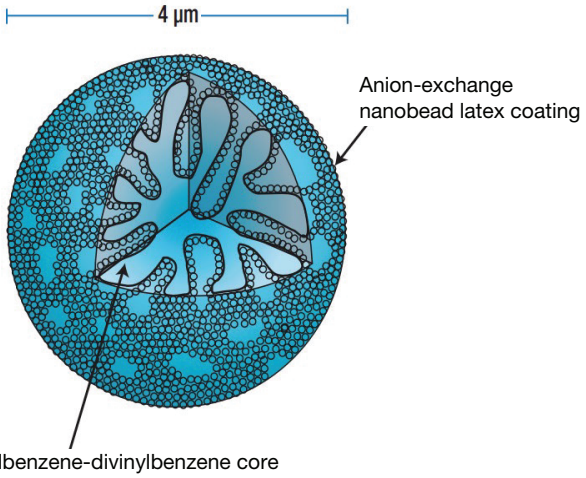


Figure 1. Structure of a Dionex CarboPac PA300-4µm column resin particle.

Dionex CarboPac PA300-4µm columns are stable over the entire pH range from 0-14. The pH stability of this packing material allows eluent compositions that are conducive to anodic oxidation of carbohydrates at the surface of gold electrodes. Note that the use of small resin particles results in higher backpressures, therefore, the Dionex CarboPac PA300-4µm columnn requires the use of high-pressure IC (HPIC™) systems such as the Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ system or ICS-6000 HPIC™ system.

Simple, direct approach using pulsed electrochemical detection

The Dionex CarboPac PA300-4µm column has been developed for efficient separation of simple and complex carbohydrates, both neutral and charged, without compromising resolution. Electrochemical detection is used to measure the current resulting from oxidation or reduction of analyte molecules at the surface of a working electrode. During oxidation reactions, electrons are transferred from molecules of electroactive analytes, such as carbohydrates, to the working electrode in the electrochemical cell. Detection is sensitive and highly selective for electroactive species; many potentially interfering species cannot be oxidized or reduced at the specified conditions and are therefore not detected.

When a single potential is applied to the working electrode, the detection method is DC amperometry. PAD, which uses a repeating sequence of potentials, is used for carbohydrate analysis and is a reproducible and sensitive method for the detection of most carbohydrates.

Analysis of sugar alditols

Many biomolecules are adorned with glycan post-translational modifications, and these sugars can be diverse and heterogeneous in composition. Many of these glycans are known to play key roles in biology, often changing the function of a biomolecule dramatically. Therefore, it is essential to identify and characterize the glycan components of a system to understand it fully. Toward this end, monosaccharides and glycan components are frequently released from biomolecules and reduced into their sugar alcohol forms. As shown in Figure 2 below, using the Dionex CarboPac PA300-4µm and 10 mM NaOH, seven sugar alditols can be separated within 12 min.

Analysis of free oligosaccharides from bovine milk

Oligosaccharides are bioactive molecules that have a variety of health benefits to consumers. One of the known health benefits is the use of oligosaccharides as prebiotics, which play an important role in establishing the intestinal microbiota by selectively simulating the growth of beneficial bacteria. Bovine milk contains oligosaccharides that have structures similar to those found in human milk, and there is a growing interest in examining the structures of those milk oligosaccharides to understand their potential biological benefits. Figure 3 shows the separation of oligosaccharides from a commercial, lactose-free bovine milk sample after sample cleanup with a Thermo Scientific™ HyperSep™ Hypercarb™ Filter Plate. The chromatogram clearly shows the ability of the CarboPac PA300-4µm column to separate charged, sialylated glycan structures from neutral, non-sialylated structures.

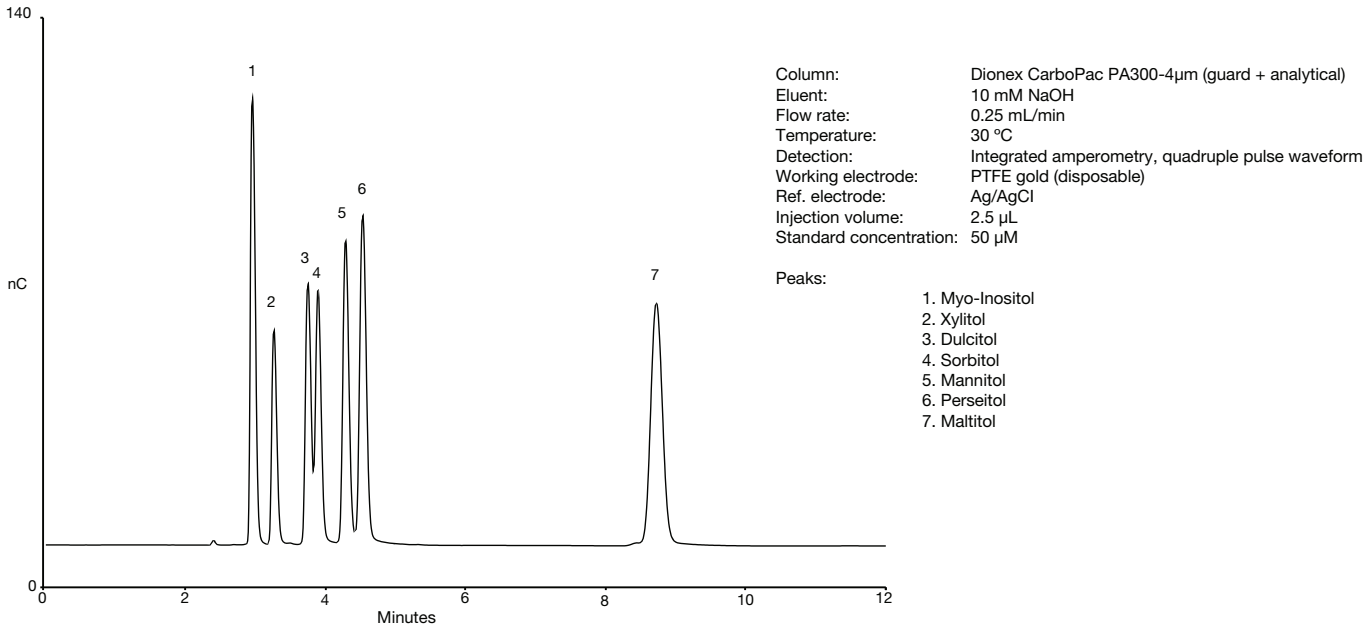


Figure 2. Separation of seven sugar alditol standards using a Dionex CarboPac PA300-4µm column.

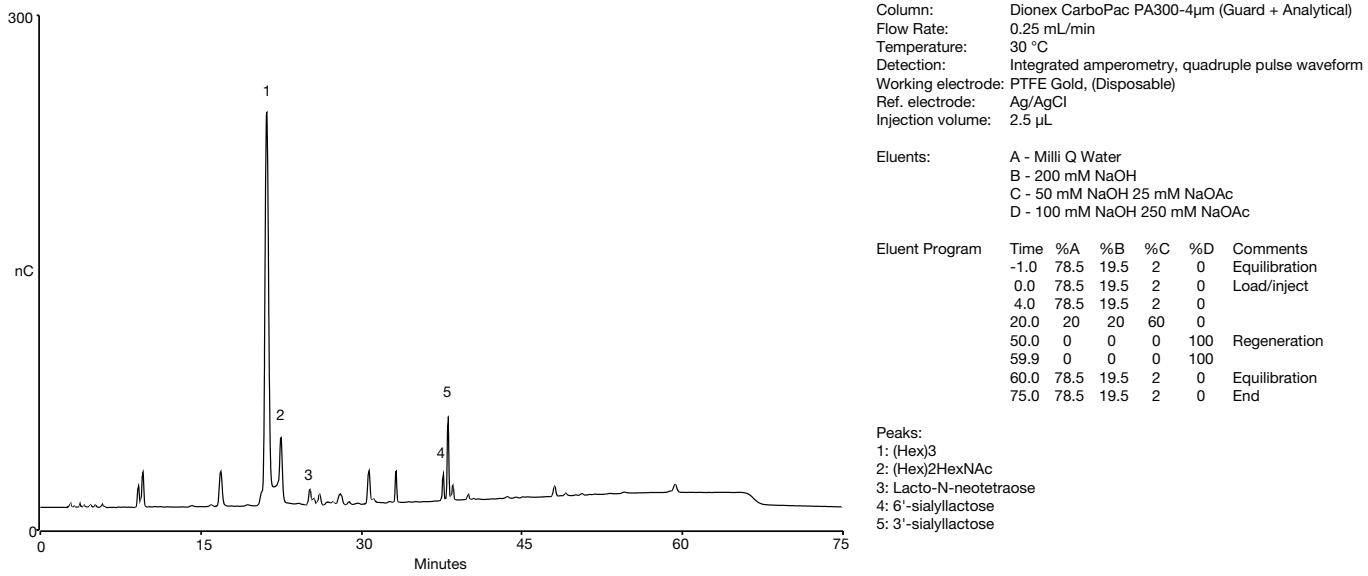


Figure 3. Chromatogram of free oligosaccharides from commercial lactose-free milk using a CarboPac PA300-4µm column.

Analysis of porcine gastric mucin

Glycosylation in mammals represents a very diverse set of pre- and post-translational modifications. One of the most diverse and complex sets of glycans is found on mammalian mucin proteins. When the CarboPac PA300-4µm column is used in an HPAE-MS workflow, it takes advantage of the improved chromatographic separation as well as the high resolution and mass accuracy of the orbitrap mass analyzer, therefore enabling the identification of glycan structures.

Figure 4 shows a schematic of a possible HPAE-PAD/MS platform configuration. Two pumps are used — one for the eluent system for separation, and another for water regeneration of the downstream ERD-500 Desalter. Following the CarboPac PA300-4µm column, a splitter can be used to divert flow to the electrochemical detector and the desalter for subsequent MS analysis. The high resolution, fragmentation-rich MS^2 spectra obtained with the Thermo Scientific™ Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap™ Mass Spectrometer provide essential glycan structural information.

High-resolution separations, especially to separate populations of chemically similar but structurally heterogenous glycans, becomes important for subsequent mass spectrometric characterization. Figure 5 demonstrates the base peak chromatogram of porcine gastric mucin type III O-glycans separated with a Dionex CarboPac PA300-4µm column and detected with a Thermo Scientific™ Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap™ Mass Spectrometer. Here, we show that the CarboPac PA300-4µm column resolves neutral and charged (i.e. sialylated and sulfated glycans) O-linked glycans from a porcine gastric mucin protein sample after sample cleanup with a HyperSep Hypercarb Filter Plate and without derivatization. In this example, the system was configured without a splitter, and the column flow (0.25 mL/min) was sent to the mass spectrometer, after passing through the Thermo Scientific™ Dionex™ ERD™ 500 electrolytically regenerated desalter where sodium and hydroxide ions were removed from eluting glycans. A selection of peaks are numbered and identified below in Table 1. Peak compositional identities were confirmed with previously published data[1]. Figure 6 shows characteristic chromatogram and demonstrates the ability for the CarboPac PA300-4µm to resolve compositional isomers.

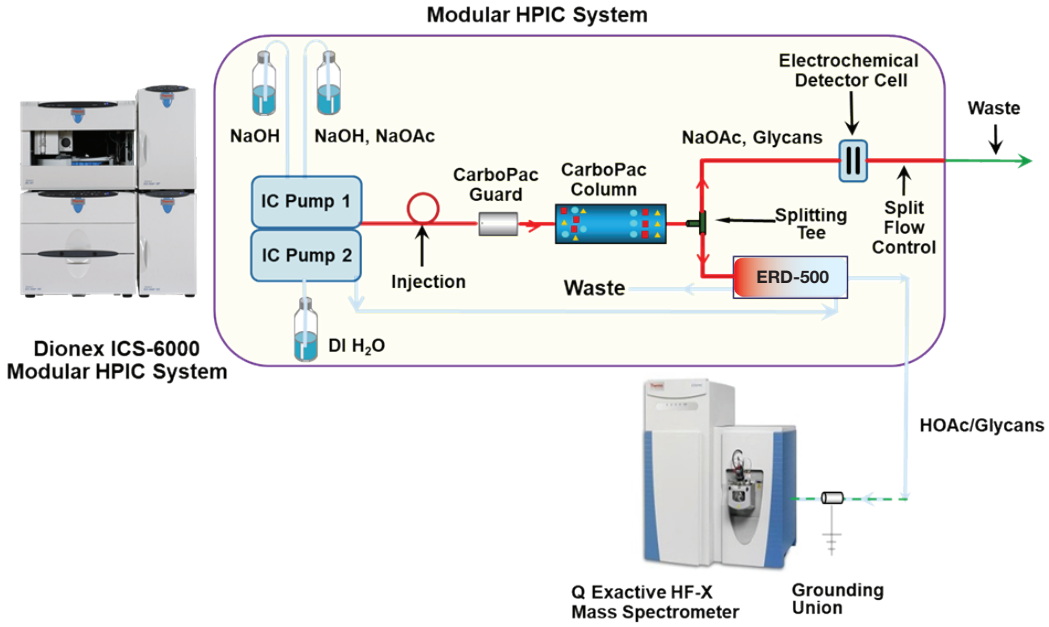
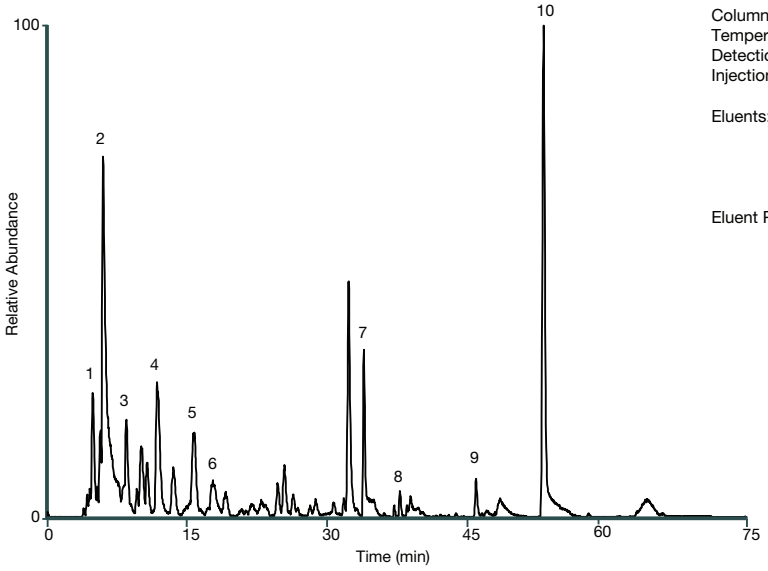


Figure 4. Schematic of a possible HPAE-PAD/MS platform configuration.



Column: Dionex CarboPac PA300-4µm (Guard + Analytical)
Temperature: 30 °C
Detection: Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap™ MS
Injection Volume: 20 µL

Eluents:
A - Milli Q Water
B - 200 mM NaOH
C - 50 mM NaOH 25 mM NaOAc
D - 100 mM NaOH 250 mM NaOAc

Eluent Program

Time	%A	%B	%C	%D	Comments
-1.0	78.5	19.5	2	0	Regeneration
0.0	78.5	19.5	2	0	
4.0	78.5	19.5	2	0	Equilibration
20.0	20	20	60	0	Load/inject
50.0	0	0	0	100	End
59.9	0	0	0	100	
60.0	78.5	19.5	2	0	
75.0	78.5	19.5	2	0	End

Figure 5. Base peak chromatogram of porcine gastric mucin type III O-glycans separated with a Dionex CarboPac PA300-4µm column and detected with a Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap™ Mass Spectrometer.

Table 1. Glycan identities of the labeled peaks in Figure 5.

Peak	RT (min)	Observed *m/z*	Theoretical *m/z*	Mass accuracy (ppm)	Composition	Ion
1	4.85	733.2896	733.2884	1.6465	HexFuc(HexNAc)2	$[M-H]^-$
2	6.08	530.2080	530.2090	1.8860	HexFucHexNAc	$[M-H]^-$
3	8.46	733.2892	733.2884	1.0910	HexFuc(HexNAc)2	$[M-H]^-$
4	11.97	1041.4006	1041.3991	1.4403	(Hex)2(Fuc)2(HexNAc)2	$[M-H]^-$
5	15.58	1098.4233	1098.4206	2.4581	(Hex)2Fuc(HexNAc)3	$[M-H]^-$
6	17.66	1203.4506	1203.4520	1.1633	(Hex)3(Fuc)2(HexNAc)2	$[M-H]^-$
7	34.01	821.3063	821.3045	2.1916	NeuAcHexFucHexNAc	$[M-H]^-$
8	38.19	852.2811	852.2848	4.3413	N-glycan, hybrid, sulfated	$[M-2H]^{2-}$
9	45.92	1121.3582	1121.3560	1.9619	(Hex)2(Fuc)2(HexNAc)2-S	$[M-H]^-$
10	53.19	813.2474	813.2452	2.7052	HexFuc(HexNAc)2-S	$[M-H]^-$

Hex: Hexose; Fuc: Fucose; HexNAc: N-Acetyl-Hexosamine; NeuAc: N-Acetylneuraminic acid; S: Sulfated

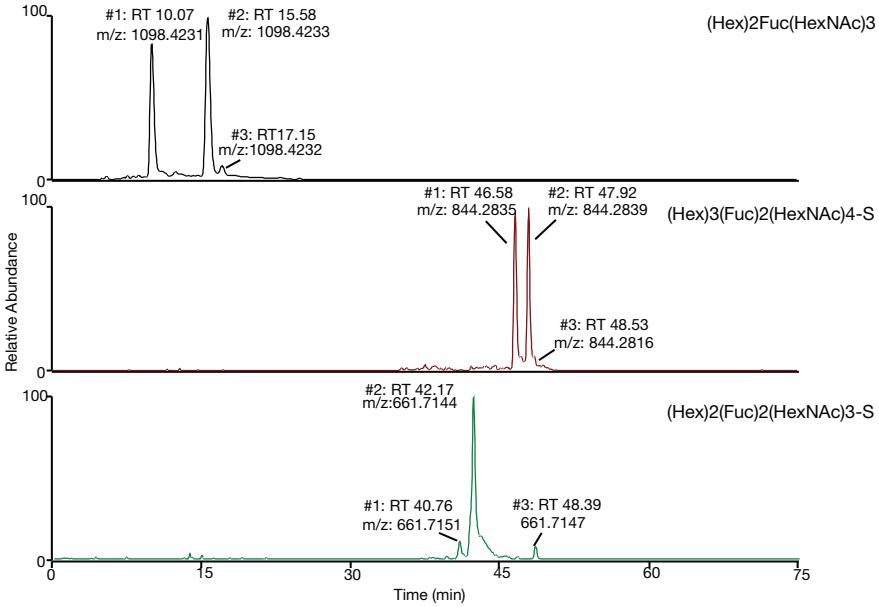


Figure 6. The CarboPac PA300-4µm column provides isomer resolution of both neutral and sulfated O-glycans from a porcine gastric mucin type III sample.

Disposable gold electrodes

Although carbohydrates can be oxidized at a gold working electrode, over time the products of the oxidation reaction foul it's surface, inhibiting further analyte oxidation.

Electrode fouling with oxidation byproducts or sample components may reduce response, requiring polishing to restore the surface. Thermo Scientific™ Dionex™ disposable gold electrodes (Figure 7) eliminate the need for electrode reconditioning. Disposable electrodes are economical, and thus, they can be replaced frequently. Frequent replacement of working electrodes renders electrochemical detection more predictable and reproducible.

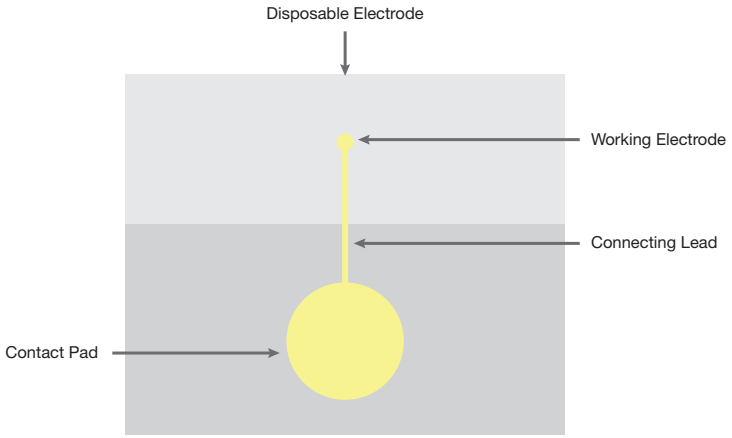


Figure 7. Representation of a disposable gold electrode.

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Ordering information

Description	Part number
Thermo Scientific Dionex CarboPac PA300-4µm Columns	
Dionex CarboPac PA300-4µm Guard Column (2x50 mm)	303347
Dionex CarboPac PA300-4µm Analytical Column (2x250 mm)	303346
Inline Trap Column	
Dionex BorateTrap Inline Trap Column	047078
Electrochemical Cells and Electrodes	
Electrochemical Detector (ED) without Cell	072042
ED with Reference Electrode and Spacer Block (no working electrode)	072044
Gold on PTFE Disposable Electrode, 6/pk, including 6 gaskets (0.002 in.)	066480
Gold Conventional Working Electrode	079850
pH-Ag/AgCl Reference Electrode	061879
Palladium Hydrogen Reference Electrode (PdH)	072075
Mass Spectrometry Components and Sample Preparation	
ERD 500, Dionex ERD 500 Electrolytically Regenerated Desalter, 2mm	085089
HyperSep Hypercarb Filter Plate, 40 µL bed volume	60110-504

Reference

1. Jin, C., Kenny, D. T., Skoog, E. C., Padra, M., Adamczyk, B., Vitizeva, V., Thorell, A., Linden S. K., & Karlsson, N. G. (2017). Structural diversity of human gastric mucin glycans. Molecular & Cellular Proteomics, 16(5), 743-758.

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