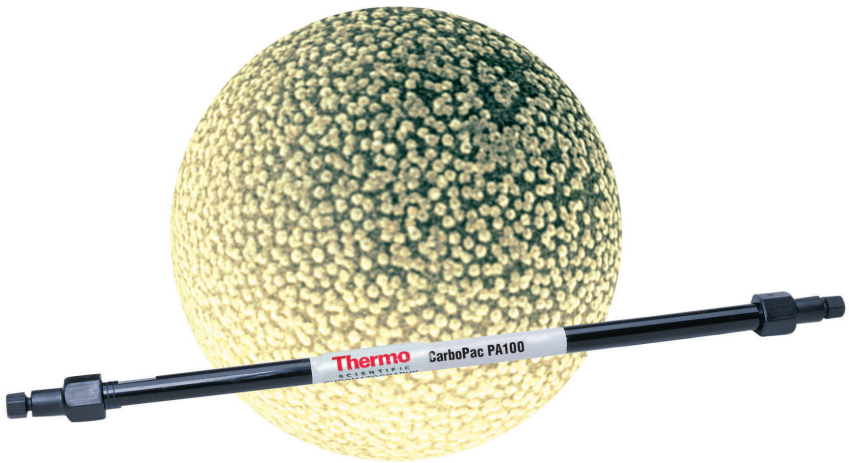
Thermo Scientific Dionex CarboPac PA100 Column

HPLC Columns for Oligosaccharide Mapping and Purification

- Predictable, high resolution separations of oligosaccharides released from glycoproteins
- Neutral and sialylated *N*-linked oligosaccharides from glycoproteins
- Oligosaccharides with monosaccharide linkage isomerism
- Oligosaccharides in food products
- Linear polysaccharide profiling
- Thermo Scientific™ Dionex™ ERD™ 500 Electrolytically Regenerated Desalter for fraction collection



Predictable, High Resolution Separations of Oligosaccharides Released from Glycoproteins

Characterization of oligosaccharides released from glycoproteins is an important but non-trivial task for the biotechnology and pharmaceutical industries. The Thermo Scientific™ Dionex™ CarboPac™ PA100 column provides the resolution needed for routine analysis of these oligosaccharides. Neutral and charged oligosaccharides are separated in their anionic forms by high-performance anion-exchange chromatography (HPAE) using the Dionex CarboPac PA100 column. Detection of these compounds at low picomole levels has been optimized using pulsed amperometric detection (PAD). No sample derivatization is required for detection.

Separations of oligosaccharides are based on their fine structural differences, such as the composition and the sequence of the oligosaccharides, linkage isomerism, degree of sialylation, and degree of branching.

There are several factors affecting the elution of oligosaccharides using the CarboPac PA100 column. Twelve empirical relationships between oligosaccharide structure and chromatographic retention are documented by Rohrer (Rohrer, *J. Glycobiology*, **1995**, 5, 359-360). Some of these are illustrated in Figure 1 and include:

- Fucosylated oligosaccharides are eluted ahead of their afucosylated analogs (peaks 1 and 2).

Thermo SCIENTIFIC

- Retention times of high mannose oligosaccharides increase as the number of mannose residues increases (peaks 2, 5, 9).
- As the degree of branching increases, the retention time of the oligosaccharide increases (peaks 8, 9, 11).
- Removal of the terminal galactose residues from a complex oligosaccharide reduces its retention time (peaks 7–9, 4–8).

Neutral and Sialylated *N*-linked Oligosaccharides from Glycoproteins

The elution of acidic sugars from the Dionex CarboPac PA100 column requires stronger eluents than those used for the elution of neutral sugars. This is usually accomplished with the addition of sodium acetate to the sodium hydroxide eluent. Sodium acetate accelerates the elution of strongly bound species and offers further selectivity control, without interfering with pulsed amperometric detection. Figures 1 and 2 show the separation of neutral and sialylated oligosaccharides in a single run. The neutral sugars are eluted in a group at the beginning of the profile followed by the disialylated, the trisialylated, and finally the tetrasialylated oligosaccharides.

Oligosaccharides with Monosaccharide Linkage Isomerism

High resolution separations can be obtained based on linkage isomerism, which is difficult to achieve using other chromatography technologies. Under alkaline conditions, the technique resolves these species not only by sialic acid content, but also according to the combination of α(2,3)- and α(2,6)-linked sialic acids within each charge class. Oligosaccharides with the greatest proportion of α(2,6)- to α(2,3)-linked sialic acids are the least retained (Figure 3). The neutral component of the oligosaccharides also influences separation: those containing a Galβ(1,3)GlcNAc sequence are retained longer than those with Galβ(1,4)GlcNAc.

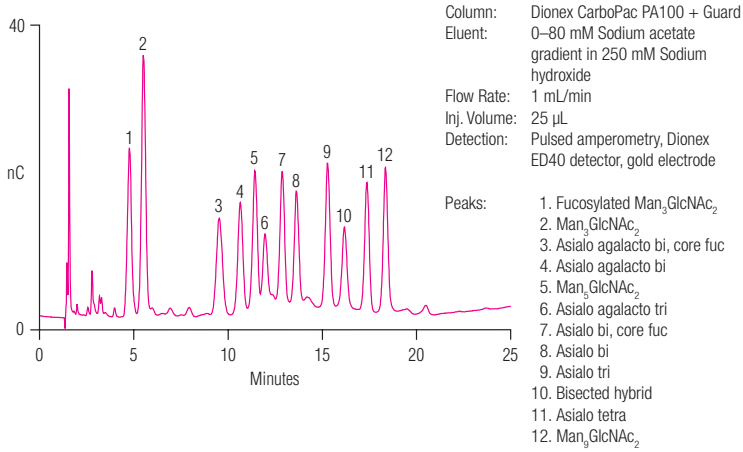


Figure 1. Separation of neutral oligosaccharide standards. Twelve commonly occurring *N*-linked neutral oligosaccharides are easily resolved within 20 minutes.

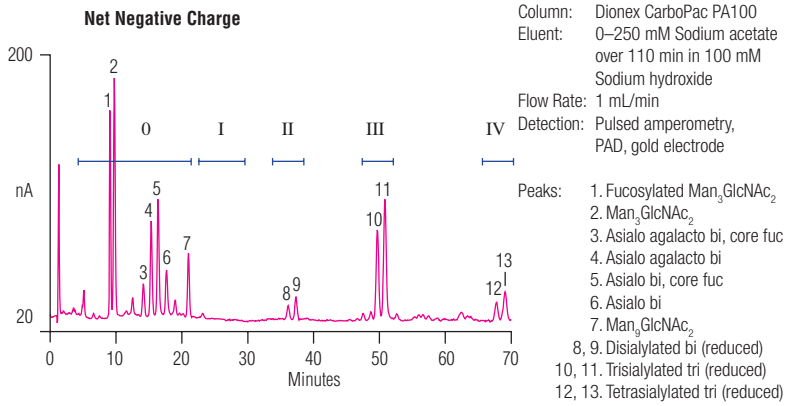


Figure 2. Separation of neutral and sialylated oligosaccharide standards. This is a commonly used method to obtain an overall profile of neutral and sialylated *N*-linked oligosaccharides released from glycoproteins. Oligosaccharides are separated into broad classes depending on their degree of sialylation. Based on the retention time windows of the oligosaccharide peaks, the degree of sialylation of the oligosaccharides can be predicted.

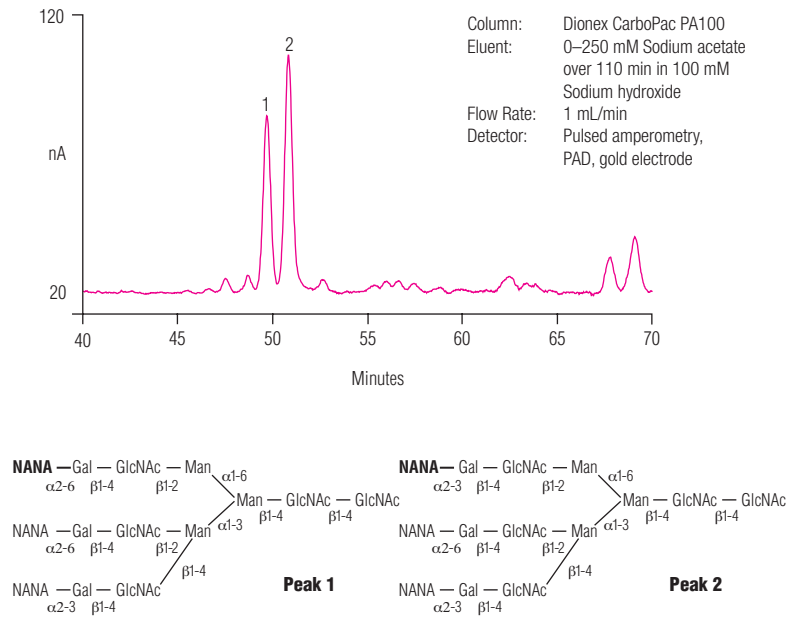


Figure 3. Separation of trisialylated oligosaccharides. The high resolving power of the Dionex CarboPac PA100 column is demonstrated by the resolution of these two oligosaccharides, which differ by only a single linkage—alpha 2-6 vs. alpha 2-3.

Oligosaccharides in Food Products

Oligosaccharides are routinely determined in food and beverage products for a variety of purposes, including quality control, verifying food-labeling claims, establishing product authenticity, and monitoring fermentation processes. Oligosaccharide profiles obtained using HPAE-PAD with the Dionex CarboPac PA100 column can be used to establish the "fingerprint" of food samples. Suspect samples can be analyzed and compared to the known profiles. This technique is useful in detecting adulteration and in quality control. For example, oligosaccharide profiles are used to detect adulteration of natural fruit juices (Figure 4), establish the geographic origin of molasses, and analyze polysaccharides in hydrolyzed glucose syrup.

The Dionex CarboPac PA100 column easily separates mono-, di-, and trisaccharides in citrus and sunflower honey (Figure 5). These profiles are used as "fingerprints" for quality control and labeling verification purposes.

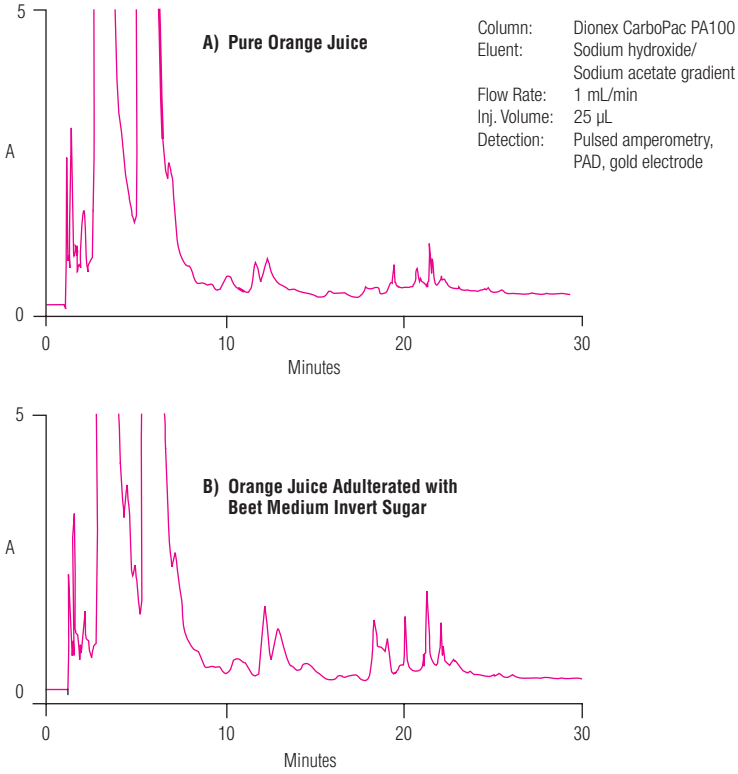


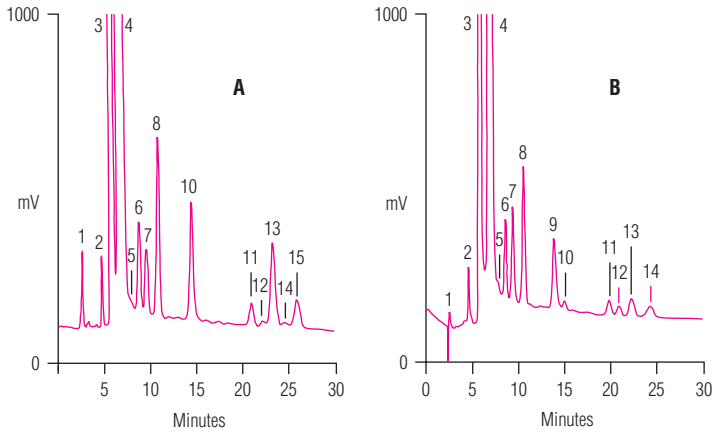
Figure 4. Oligosaccharide profiles of pure and adulterated orange juice. Oligosaccharide composition profiles can be used to detect the adulteration of natural fruit juices by inexpensive sweeteners such as beet sugar.

Column: Dionex CarboPac PA100 (4 × 250 mm) + Guard
Eluent: A: 600 mM NaOH
B: 0.5 M NaOAc
C. 18 mΩ DI Water
Flow Rate: 0.7 mL/min
Detection: Pulsed amperometry, PAD, gold electrode

Gradient:

t	A	B	C
0	15	0	85
5	15	0	85
5.1	15	2	83
12	15	2	83
12.1	15	4	81
20	15	4	81
20.1	20	20	60
30	20	20	60

Peaks:
1. Glycerol
2. α,α-Trehalose
3. Glucose
4. Fructose
5. Melibiose
6. Isomaltose
7. Sucrose
8. Unknown
9. Turanose
10. Palatinose
11. Nigerose
12. Maltose/1-Kestose
13. Laminaribiose
14. Eriose
15. Panose



Seminars in Food Analysis, Vol. 2 No. 1/2, 1997, Corrandini, C. et. al., "Application of HPAE-PAD to Carbohydrate Analysis in Food Products and Fruit Juices", Rapid Science LTD.

Figure 5. Chromatograms of di- and trisaccharides in honey from (A) citrus and (B) sunflower.

Linear Polysaccharide Profiling

Separations of high mannose, hybrid, and complex oligosaccharides are best accomplished using the Dionex CarboPac PA100 column. Separations are accelerated and improved by using sodium acetate gradients in sodium hydroxide. Figure 6 shows the gradient separation of chicory inulin, illustrating the high resolution possible with this column.

Determining the levels of fermentable and nonfermentable sugars at every stage of beer production is important because fermentable sugars determine the final alcohol content, and nonfermentable sugars contribute to the flavor and "body" of the final product. A separation of maltose oligomers up to degree of polymerization (DP) 10 is shown in Figure 7, panel A; panels B, C, and D show sugar and polysaccharide profiles at various stages of the brewing process. All samples were diluted 1:10.

Column: Dionex CarboPac PA100
Eluent: A: 100 mM NaOH
B: 100 mM NaOH with 1 M NaOAc
Flow Rate: 1.0 mL/min
Detector: Pulsed amperometry, PAD, gold electrode
Range: 1000 nA full scale

Sample: 25 µL of 0.3%(w/v) solution
Gradient:

t	A	B
0	88	11
85	45	55
87	88	12

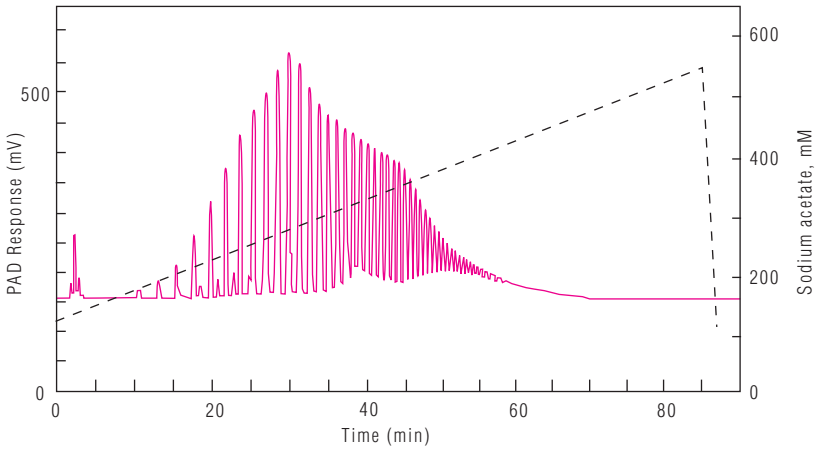


Figure 6. Gradient separation of chicory inulin using the Dionex CarboPac PA100.

Column: Dionex CarboPac PA100 and Guard
Eluent: Sodium hydroxide/ Sodium acetate gradient
Flow Rate: 1.0 mL/min
Inj. Volume: 10 µL
Detection: Pulsed amperometry, Dionex ED40 detector, gold electrode

Peaks:
1. Ethanol
2. Glucose
3. Maltose
4. Maltotriose
5–11. Maltose-Oligomers

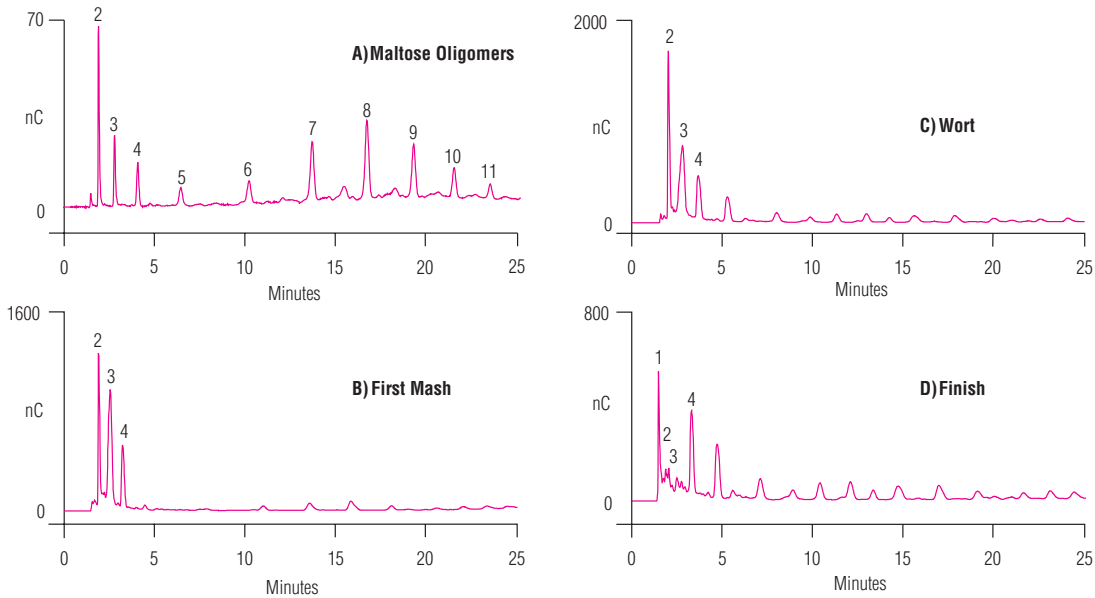


Figure 7. Sugar and oligosaccharide profiles during beer production. In panel A, maltose oligomers are baseline-separated. Chromatograms B, C, and D illustrate sugar and oligosaccharide profiles at different stages of the brewing process.

Fraction Collection

The Dionex ERD 500 Desalter device is a membrane device designed to desalt and reduce the pH of the samples following HPAE-PAD when the user wishes to collect and further analyze the carbohydrate samples. Desalted samples are then ready for lyophilization without dialysis.

More than 99% of the sodium ions in eluents that contain up to 0.35 M sodium ions, flowing at a rate of 1 mL/min, are removed by the Dionex ERD 500 Desalter device. An advantage of the Dionex CarboPac PA100 column over the Dionex CarboPac PA1 column is that a lower salt concentration is required to elute the same oligosaccharide, making the Dionex CarboPac PA100 column the best choice if fraction collection with subsequent desalting is required.

Placed after the electrochemical detector, the Dionex ERD 500 Desalter device exchanges sodium ions for hydronium ions. This process changes the sodium hydroxide and sodium acetate eluents to water and dilute acetic acid immediately after leaving the detector cell. Collected fractions can then be lyophilized, leaving the pure carbohydrate sample ready for further manipulation. These samples are suitable for enzymatic and chemical digestion, NMR or mass spectrometric analysis, or further chromatographic analysis. Figure 8 shows the effect of the added volume of the Dionex ERD 500 Desalter device between detection and collection points. Loss of resolution is measurable (~6%), but chromatographic resolution is sufficient to allow acceptable purification.

Rugged, Reliable Separation with Guaranteed Performance

The unique pellicular resin of the Dionex CarboPac PA100 columns offers exceptional selectivity and stability over the entire pH range. Its highly crosslinked structure ensures long column life and easy clean-up.

The entire manufacturing process (resin synthesis, amination, and packing and testing of the chromatographic columns) is carefully controlled to ensure that every Dionex CarboPac PA100 column delivers reproducible performance. Dionex CarboPac PA100 columns are tested with two isomers of N-acetylneuraminosyl-D-lactose to ensure lot-to-lot reproducibility.

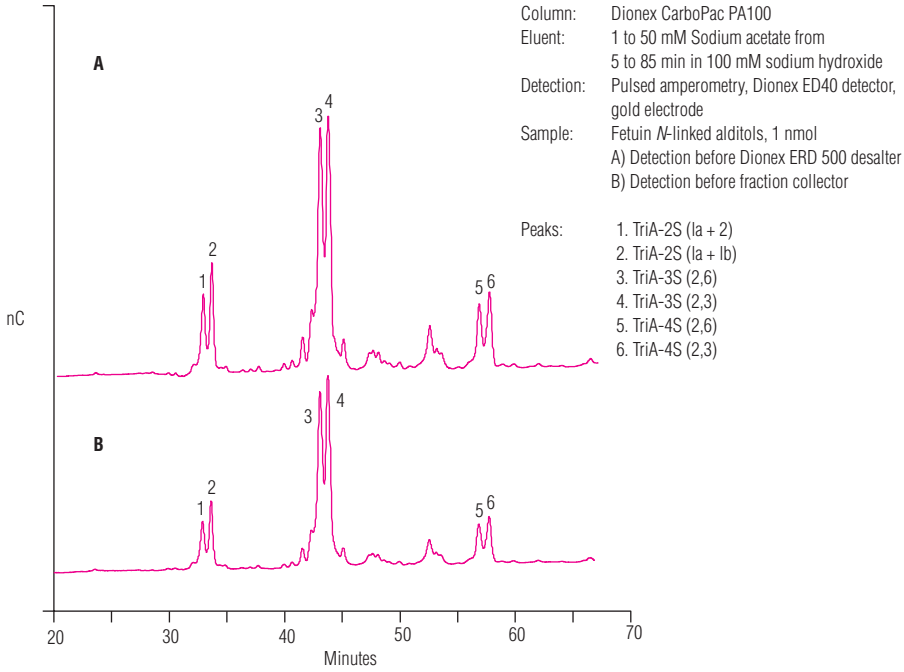


Figure 8. Elution of 1 nmol fetuin *N*-linked alditols.

SPECIFICATIONS

Dionex CarboPac PA100 Column

Resin Composition
8.5 µm diameter ethylvinylbenzene/ divinylbenzene substrate (55% crosslinking) agglomerated with 275 nm MicroBead™ 6% crosslinked quaternary amine-functionalized latex.

Anion-Exchange Capacity
Approximately 90 µeq/column (4 × 250 mm)

Maximum Operating Pressure
4000 psi (28 MPa)

Chemical Compatibility
pH 0–14, 100% compatible with common organic solvents

Temperature Range
4–90 °C

Test Procedure
Separation of a sialylated N-linked fetuin alditol test mixture (P/N 043064)

Recommended Operating Temperature
Ambient

Recommended Flow Rate
1.0 mL/min

Ionic Form Eluents
Sodium acetate and sodium hydroxide only

Ordering Information

In the U.S., call (800) 346-6390 or contact the Thermo Fisher Scientific Regional Office nearest you. Outside the U.S., order through your local Thermo Fisher Scientific office or distributor. Refer to the following part numbers. Thermo Fisher Scientific can also make special order Dionex CarboPac columns to your specifications; call for more information.

Description	Part Number
Dionex CarboPac PA100 Columns	
CarboPac PA100 Analytical Column (4 × 250 mm)	043055
CarboPac PA100 Guard Column (4 × 50 mm)	043054
CarboPac PA100 Microbore Column (2 × 250 mm)	057182
CarboPac PA100 Microbore Guard Column (2 × 50 mm)	057183
CarboPac PA100 Semipreparative Column (9 × 50 mm)	SP2089
CarboPac PA100 Preparative Column (22 × 250 mm)	SP2667
Carbohydrate Membrane Desalter	
Dionex ERD 500 Electrolytically Regenerated Desalter (4 mm)	085087
Dionex ERD 500 Electrolytically Regenerated Desalter (2 mm)	085089

www.thermoscientific.com/chromatography

Thermo Fisher Scientific, Sunnyvale, CA USA is ISO 9001 Certified.

Africa +43 1 333 50 34 0
Australia +61 3 9757 4300
Austria +43 810 282 206
Belgium +32 53 73 42 41
Brazil +55 11 3731 5140
Canada +1 800 530 8447
China 800 810 5118 (free call domestic)
400 650 5118
Denmark +45 70 23 62 60
Europe-Other +43 1 333 50 34 0
Finland +358 9 3291 0200
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9494
Italy +39 02 950 591
Japan +81 6 6885 1213
Korea +82 2 3420 8600
Latin America +1 561 688 8700
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Norway +46 8 556 468 00
Russia/CIS +43 1 333 50 34 0
Singapore +65 6289 1190
Sweden +46 8 556 468 00
Switzerland +41 61 716 77 00
Taiwan +886 2 8751 6655
UK/Ireland +44 1442 233555
USA +1 800 532 4752

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand

PS71533-EN 0315S