ProPac® Ion Exchange Columns for Protein Analysis



Ion-Exchange Columns for Characterization and Quality Control Assessment of Closely Related Protein Variants:

- Sequence
- Truncation
- · Phosphorylation
- Sialylation
- Deamidation

Now sold under the Thermo Scientific brand



Unique Chemistry for High-Resolution Analysis of Proteins with Small Differences in Charge

Dionex developed the ProPac line of ion-exchange columns specifically to provide high-resolution, high-efficiency separations of proteins and glycoproteins (pI = 3-10; MW > 10,000). The rigid, nonporous pellicular resin provides exceptionally high resolving power, permitting the separation of proteins that differ by as little as one charged residue. Hydrophobic interactions with the resin are eliminated for very efficient peaks.

ProPac columns are designed to address the regulatory requirements for biopharmaceutical characterization. The reproducible resin chemistry and manufacturing processes eliminate column variability as a concern in methods development and data analysis.

ProPac columns are available with weak or strong anion-exchange or cation-exchange resins packed in 2-, 4-, 9-, and 22-mm i.d. formats. The selectivity differences between the various resin types provide flexibility in maximizing the resolution of closely related proteins.



Sequence Variants

Clinical assays of hemoglobin sequence variants are important in the diagnosis of hemoglobinopathies. The strong cation-exchange resin of the ProPac SCX-10 produces a rapid, high-resolution separation of hemoglobin variants. Figure 1 shows several commonly observed hemopathic hemoglobins. The high rate of mass transfer associated with the pellicular resin affords efficient peaks and high resolution.

Truncation Variants (MAbs)

The weak cation-exchange packing of the ProPac WCX-10 is a unique resin with a hydrophilic coating and carboxylate functional groups. The physicochemical properties of this support eliminate secondary interactions between the protein analytes and the stationary phase. This results in minimum band spreading and high selectivity, as illustrated in the baseline resolution of C-terminal variants of an IgG_1 monoclonal antibody (MAb) (Figure 2).

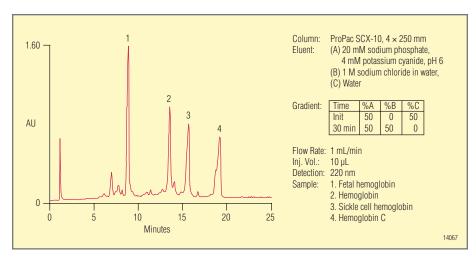


Figure 1. Separation of hemoglobin variants, including fetal (HbF), sickle cell (HbS), normal (HbAo), and hemoglobin C (HbC).

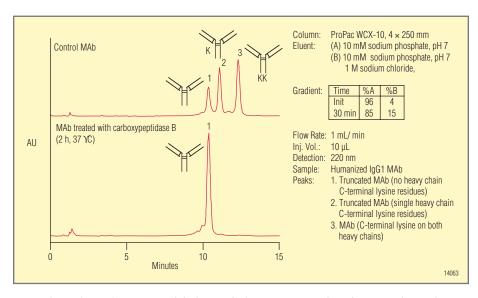


Figure 2. Analysis of an IgG_1 MAb before and after treatment with carboxypeptidase B for two hours at 37 °C.

Phosphorylation Variants

The resolution of phosphorylation variants is important in the characterization of biomacromolecules.³ The high efficiency of the strong anion-exchange resin of the ProPac SAX-10 allows for the resolution of several phosphorylation isoforms of ovalbumin using a simple linear gradient (Figure 3, bottom trace). After treatment with alkaline phosphatase, the eight resolved isoforms simplify to one major peak and three minor peaks (Figure 3, top trace).

Sialylation Variants

Published data suggest that different isoform profiles of transferrin are diagnostic of different clinical conditions.⁴ Using the ProPac SAX-10, the differences in elution profile resulting from differences in sialylation can be seen (Figure 4).⁵ When the different transferrin samples are digested with neuraminidase to remove sialic acid, the profiles collapse into a similar pattern.

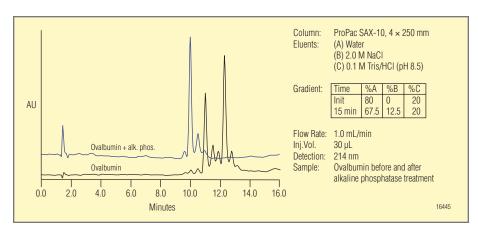


Figure 3. Resolution of phosphorylation variants of ovalbumin.

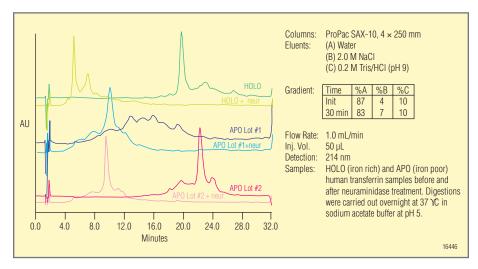


Figure 4. Effect of sialylation on transferrin chromatography.

Deamidation Variants

Determining the deamidation of asparagine (Asn) residues is a significant challenge for analytical and protein chemists in the QA and process departments at biotechnology and pharmaceutical companies. The unique selectivity of the ProPac columns allows the rapid analysis of protein deamidation with baseline resolution in a single step, as illustrated in Figure 5 for the separation of ribonuclease A and its two deamidation products. The baseline resolution allows quantification of each form of the protein as a function of time.

Unique Resin Technology

The ProPac resin technology is the basis for the superior performance of these ion-exchange columns. The thin, hydrophilic layer grafted to the particle core eliminates hydrophobic interactions with proteins (Figure 6). To this layer are grafted the unique polymer chains (containing the anion-or cation-exchange groups) that impart selectivity and reproducible capacity.

References

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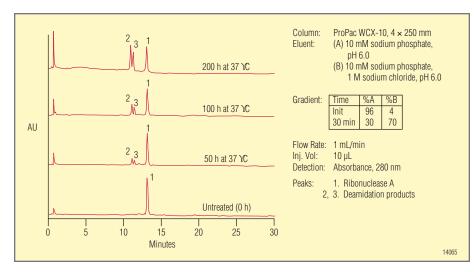


Figure 5. Separation of ribonuclease A and its two deamidation products during the course of forced deamidation. Ribonuclease A (3 mg/mL) was incubated in 1% ammonium carbonate buffer, pH 8.2, at 37°C.

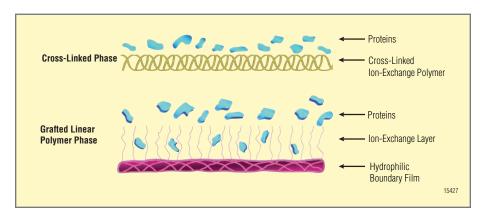


Figure 6. Protein interaction with cross-linked phase compared to grafted linear polymer phase.

SPECIFICATIONS

Maximum Operating Pressure: 21 MPa (3,000 psi)

Chemical Compatibility:

Solvents: 80% MeCN, acetone for all ProPac columns (SAX, WAX, SCX, WCX); 80% methanol for SAX, WAX, and SCX only. Alcohols are incompatible with the WCX-10.

Detergents: Nonionic and zwitterionic are compatible. Cationic detergents are incompatible with cation-exchange columns (WCX-10, SCX-10); anionic detergents are incompatible with anion-exchange columns (WAX-10, SAX-10).

pH Range: 2-12

Typical Buffers: Phosphate, acetate,

Temperature:

≤40 °C

Column Construction:

PEEK with 10–32 threaded, ferrule-style end fittings. All components are nonmetallic.

Resin Composition:

Substrate Bead Diameter: 10 µm

Substrate Composition: Ethylvinylbenzene 55% cross-linked with divinylbenzene

Pellicular Layer: Proprietary, neutral, hydrophilic

Functional Groups:

WCX-10: Carboxylate

SCX-10: Sulfonate

WAX-10: Tertiary amine

SAX-10: Quaternary ammonium

Column Capacity:

Depending on the specific proteins and resolution of the protein peaks, >100 μ g of protein can be injected on a 4 × 250 mm column without loss of resolution.

ORDERING INFORMATION

In the U.S., call 1-800-346-6390 or contact the Dionex regional office nearest you. Outside the U.S., order through your local Dionex office or distributor. Refer to the following part numbers. Dionex can also make special-order ProPac columns to your specifications; call for more information.

Description Part Num	ber
ProPac Weak Cation-Exchange Columns (Carboxylate Functionality) ProPac WCX-10 Analytical Column (4 × 250 mm)	993
ProPac WCX-10G Guard Column (4 × 50 mm)	
ProPac WCX-10 Analytical Column (2 × 250 mm)	
ProPac WCX-10G Guard Column (2 × 50 mm)	251
ProPac WCX-10 Semipreparative Column (9 × 250 mm)	480
ProPac WCX-10 Semipreparative Column (22 × 250 mm)	482
Lot select column set—Three columns from one resin lot (4 × 250 mm)	512
Lot select column set—One column from each of three resin lots (4 × 250 mm)	513
ProPac Strong Cation-Exchange Columns (Sulfonate Functionality) ProPac SCX-10 Analytical Column (2 × 250 mm)	572
ProPac SCX-10G Guard Column (2 × 50 mm)	574
ProPac SCX-10 Analytical Column (4 × 250 mm)	995
ProPac SCX-10G Guard Column (4 × 50 mm)	930
ProPac SCX-10 Semipreparative Column (9 × 250 mm)	520
ProPac SCX-10 Semipreparative Column (22 × 250 mm)	
$(4 \times 250 \text{ mm})$	
ProPac Weak Anion-Exchange Columns (Tertiary Amine Functionality) ProPac WAX-10 Analytical Column (2 × 250 mm)	558
ProPac WAX-10G Guard Column (2 × 50 mm)	560
ProPac WAX-10 Analytical Column (4 × 250 mm)054	.999
ProPac WAX-10G Guard Column (4 × 50 mm)	150
ProPac WAX-10 Semipreparative Column (9 × 250 mm)	596
ProPac WAX-10 Semipreparative Column (22×250 mm)	
Lot select column set—One column from each of three resin lots (4 × 250 mm)	

ORDERING INFORMATION

ProPac Strong Anion-Exchange Columns (Quaternary Ammonium Funct ProPac SAX-10 Analytical Column (2 × 250 mm)	- /
ProPac SAX-10G Guard Column (2 × 50 mm)	SP5556
ProPac SAX-10 Analytical Column (4 × 250 mm)	.054997
ProPac SAX-10G Guard Column (4 × 50 mm)	.054998
ProPac SAX-10 Semipreparative Column (9 × 250 mm)	SP5592
ProPac SAX-10 Semipreparative Column (22 × 250 mm)	SP5594
Lot select column set—Three columns from one resin lot (4 × 250 mm)	SP5731
Lot select column set—One column from each of three resin lots $(4 \times 250 \text{ mm})$	SP5730

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