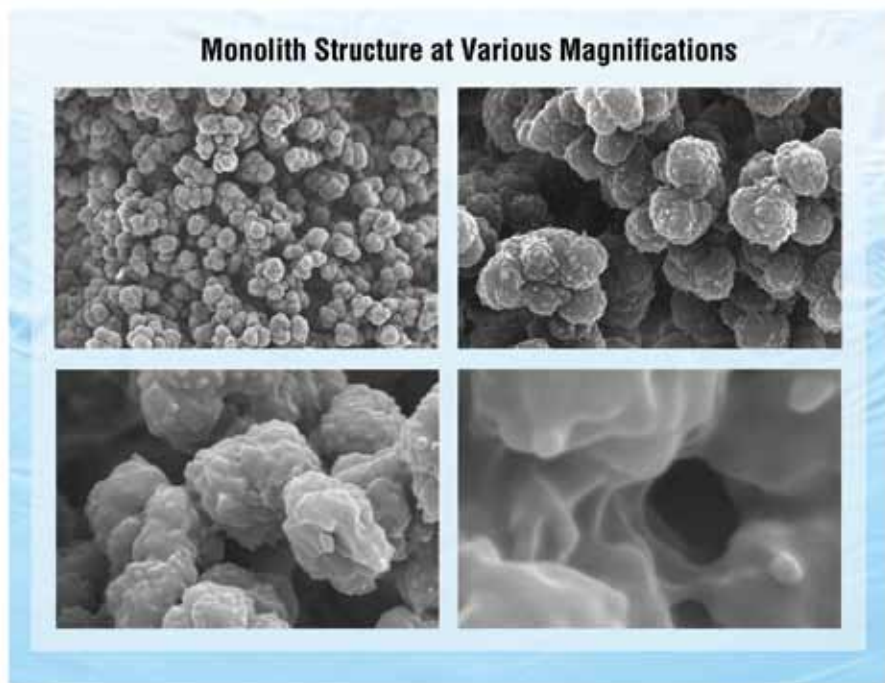


columns

ProSwift Ion-Exchange Monolith Columns for Protein Analysis



ProSwift® Ion-Exchange columns use a unique monolith technology for fast, high-resolution HPLC and LC/MS separation and purification of proteins. The columns have several advantages over traditional packed columns. ProSwift Ion-Exchange columns deliver the outstanding resolving power of nonporous media combined with the high capacity of conventional porous media. They also deliver faster separations than bead-based columns. In addition, the monolith columns exhibit stability and reproducibility even after hundreds of runs.

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Column Characteristics

ProSwift Ion-Exchange monoliths are a new family of fast, high-resolution columns for protein analysis and small-scale purification. Their unique monolith design provides:

- High resolution
- Fast mass transfer
- Fast separations
- Large loading capacity
- Wide range of operational flow rates
- Low backpressure
- Excellent stability over a wide pH range
- Outstanding reproducibility and ruggedness
- Optimal performance in a broad range of applications

ProSwift Ion-Exchange columns provide fast, high resolution protein separations using conventional HPLC. The unique monolith support is a single, cylindrical polymeric rod that offers significant advantages over traditional packed-bed media. The monolith structure is designed and engineered to contain an uninterrupted, interconnected network of channels of a specific, controlled pore size. These large flow-through channels and essentially nonporous surfaces support fast mass transfer, especially for proteins, resulting in high resolution and fast separations. These channels also produce low backpressures, allowing the use of higher linear velocities with minimal loss of resolution. Proteins can be separated and purified at high throughput for characterization studies or 2D-LC/MS analysis.



Passion. Power. Productivity.

High Resolution

ProSwift monolith columns deliver the outstanding resolving power of nonporous media with the capacity of porous beads. ProSwift columns typically provide superior resolution for proteins compared to conventional columns with porous beads. The higher resolution is achieved by a faster mass transfer, primarily driven by convective flow through the large channels and nonporous surfaces of the monolith. Mass transfer in and out of pores of conventional porous bead packing material is limited by low diffusivity and steric hindrance, resulting in peak broadening. This limitation is especially notable for large biomolecules like proteins. ProSwift columns minimize these problems. Figures 1 and 2 show comparisons between ProSwift monolith columns and porous bead columns of a leading competitor, clearly showing the superior resolution of the monolith columns.

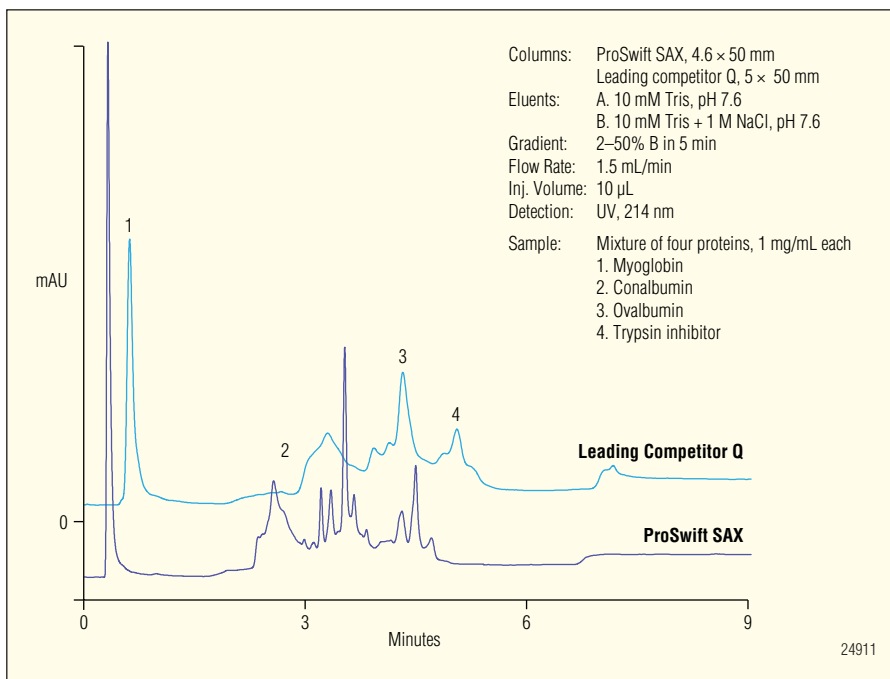


Figure 1. Comparison of ProSwift SAX-IS and leading competitor—separation of a protein mixture.

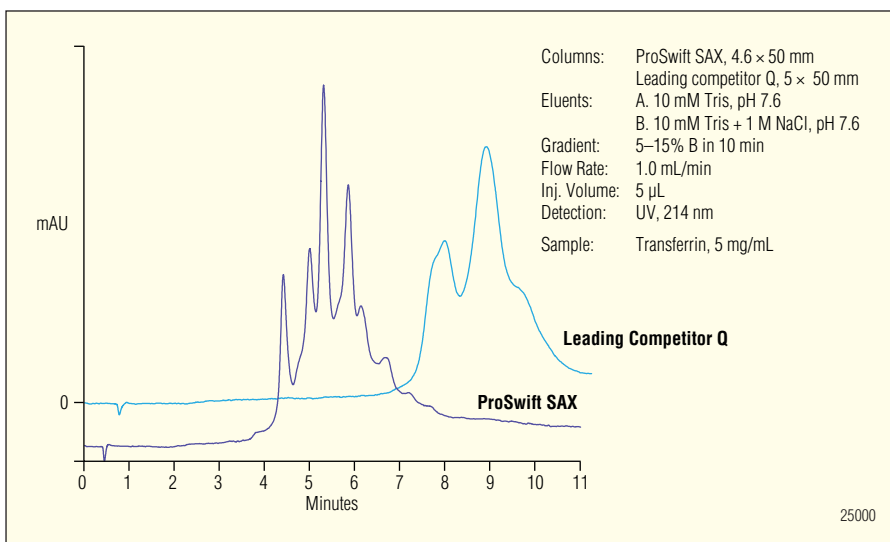


Figure 2. Comparison of ProSwift SAX-IS and a leading column competitor—separation of transferrin variants.

High Capacity

ProSwift Ion-Exchange columns provide high capacity for large biomolecules such as proteins. This high capacity is a property of the unique monolith morphology. It is achieved by flow-through channels that provide access for large biomolecules such as proteins to the irregular globular surface of the ProSwift monolith morphology. With the capacity for milligram quantities of sample, ProSwift Ion-Exchange 4.6 × 50 mm columns can be used for research and development preparative chromatography.

Even with these high loading capacities, ProSwift columns maintain high resolution. Figure 3 shows the separation of phosphorylation variants of the ovalbumin using the ProSwift SAX-1S 4.6 × 50 mm column. Increased loading of the sample, up to 640 µg, does not affect the separation of those variants.

The combination of high loading capacity and low column volume makes the ProSwift 1-mm columns an excellent choice as the first dimension in multi-dimensional separations. Figure 4 shows the high dynamic loading capacity of a 1 × 50 mm ProSwift WCX-1S monolith column. A protein mixture containing up to 72 µg of a protein mixture was injected, and the corresponding peak widths at half heights were compared. The comparison clearly shows that increasing sample loads result in only minor increases in peak width (Figure 4 inset).

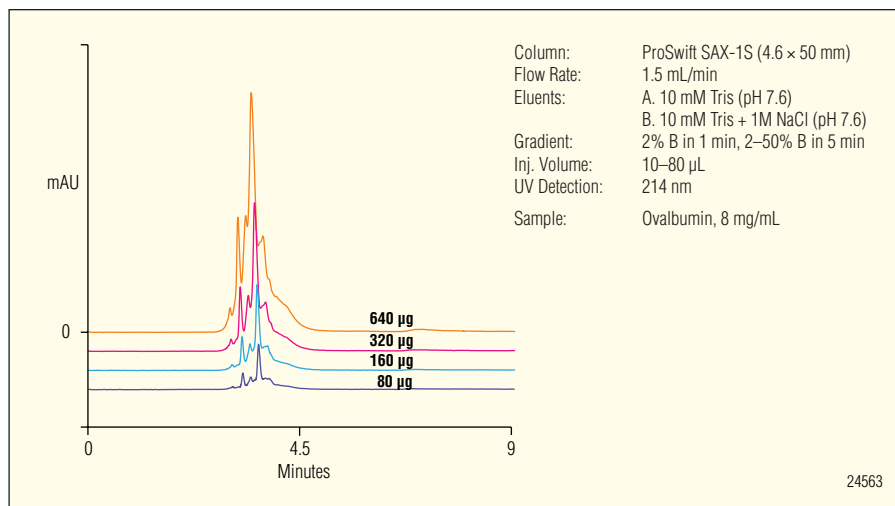


Figure 3. Separation of phosphorylation variants of ovalbumin using the ProSwift SAX-1S 4.6 × 50 mm column.

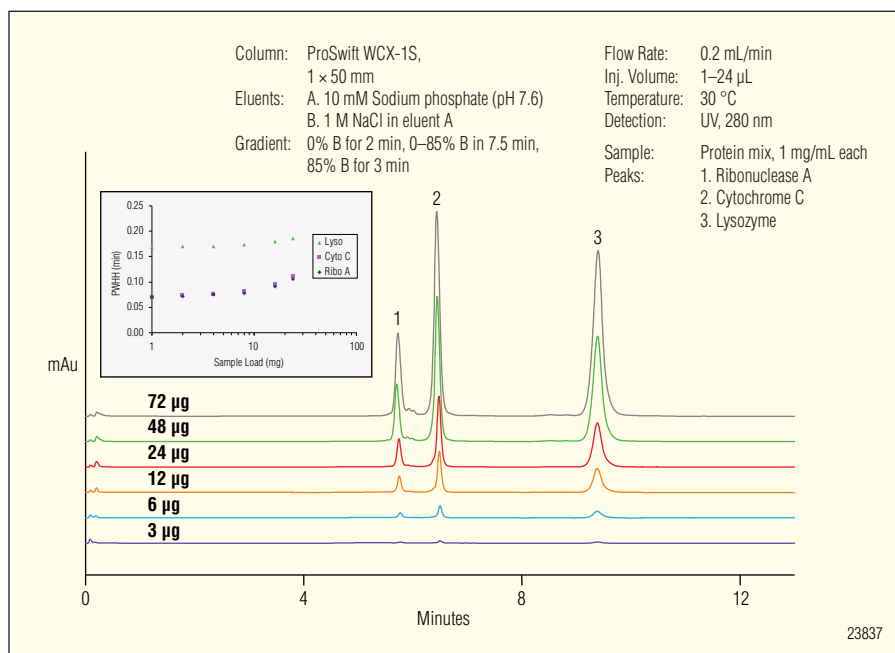


Figure 4. Dynamic protein loading capacity of WCX-1S 1 × 50 mm.

Ruggedness and Reproducibility

ProSwift columns are durable and robust. They exhibit stability and reproducibility even after hundreds of runs. Figure 5 shows ProSwift SAX-1S retaining high resolution for over 800 cycles.

Column Diversity

ProSwift Ion-Exchange columns are available in a variety of formats for the separation, purification and analysis of proteins. The Ion-Exchange monolith family includes both strong and weak anion-exchange phases as well as strong and weak cation-exchange phases. The different phases provide a variety of selectivities to choose from. Figure 6 shows different selectivities between ProSwift SCX-1S and WCX-1S 1 x 50 mm columns. ProSwift Ion-Exchange columns are available in 4.6 x 50 mm or 1.0 x 50 mm formats. Figure 7 shows protein separations using ProSwift SCX-1S columns in different formats. (See specification table and ordering information.)

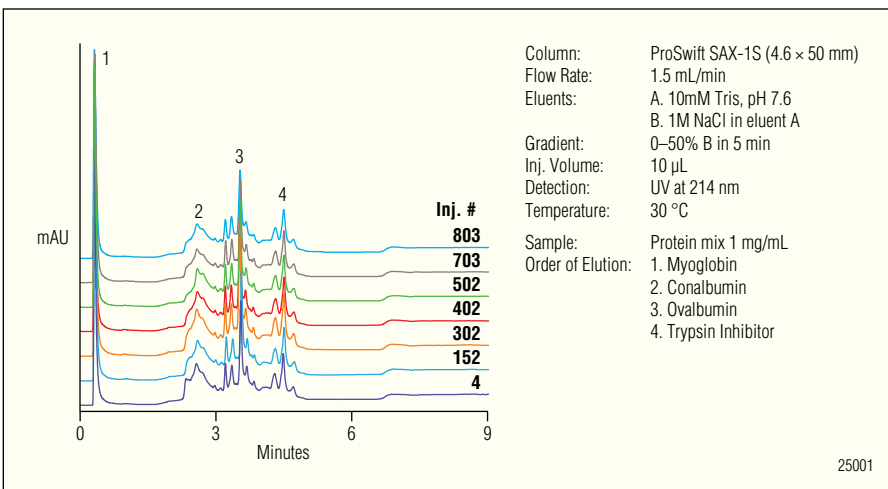


Figure 5. Ruggedness and reproducibility of the ProSwift SAX-1S.

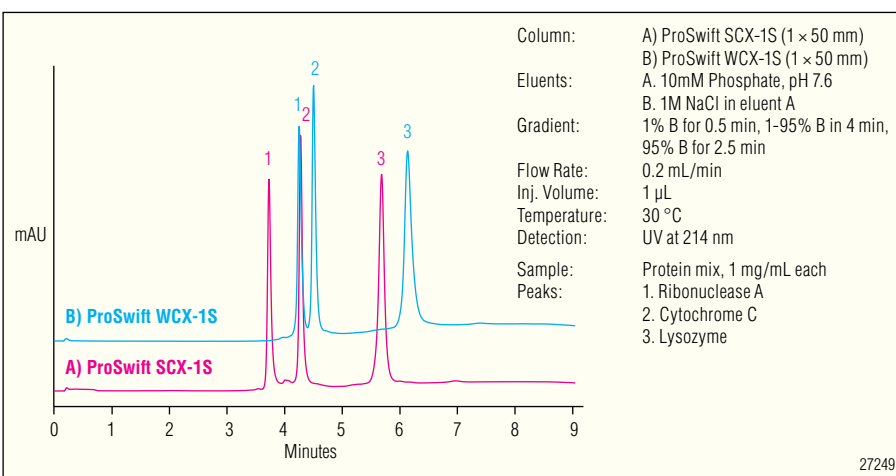


Figure 6. Comparison of the different selectivities between ProSwift SCX-1S and WCX-1S 1 x 50 mm columns.

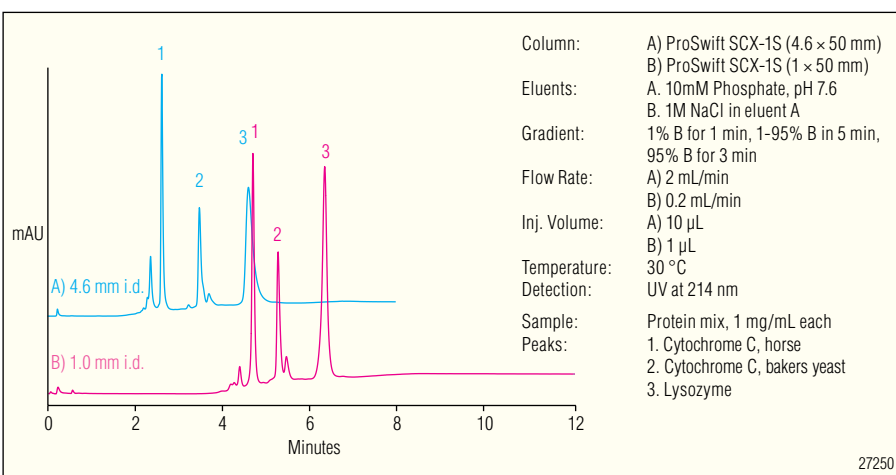


Figure 7. Protein separations using ProSwift-SCX-1S columns in different formats.

Wide Range of Applications

ProSwift Ion-Exchange monolith columns yield optimal performance in a wide range of applications, including:

- Complex protein samples
- Upstream LC/MS first-dimensional protein fractionation
- Analytical HPLC
- Preparative HPLC

Specific examples follow.

Separation of Complex Proteins in Supernatant and Cell Lysate

Figure 8 shows the high resolving power of the ProSwift SAX-1S 4.6 x 50 mm monolith column. Here, it is used to separate a complex protein sample of pancreatin supernatant.

The analysis of highly-complex protein mixtures often requires multi-dimensional chromatography. For top down proteomics, the use of high-resolution, high-capacity columns in the first dimension is an early step in identifying individual proteins. Figure 9 illustrates the high resolution and capacity of the ProSwift WAX-1S 1.0 x 50 mm column. The data show that the column is ideal for separating a complex mixtures of proteins, in this case, obtained from the lysate of *Escherichia coli* cells.

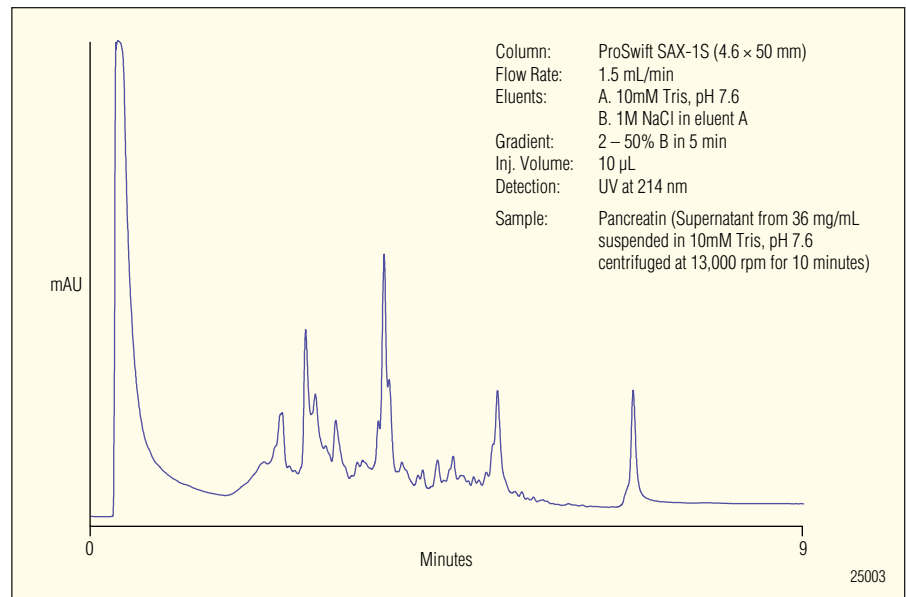


Figure 8. Separation of pancreatin on ProSwift SAX-1S column.

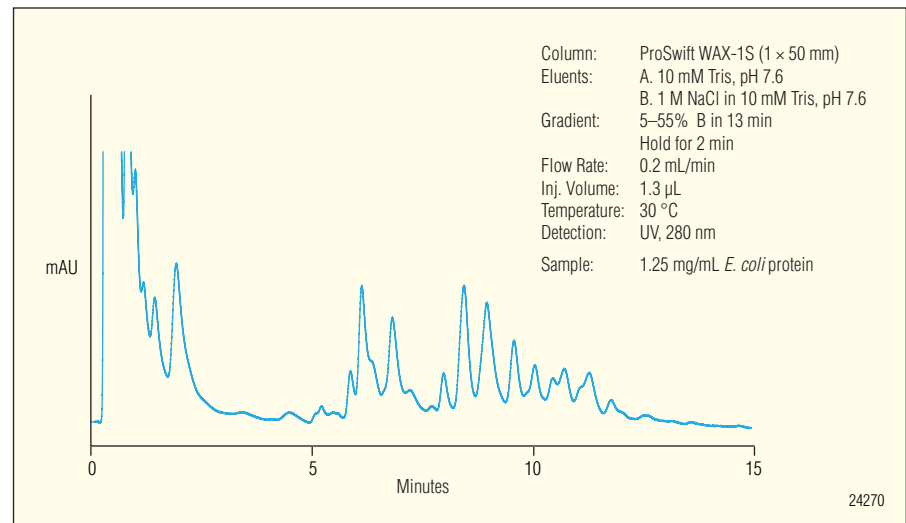


Figure 9. Separation of *E. coli* proteins using the ProSwift WAX-1S.

Determination of MAb Variants

Monoclonal antibody (MAb) microheterogeneity, including C-terminal processing of lysine residues on the heavy chains, is a common structural variation that demands careful analysis. As a result of this processing, C-terminal lysine or arginine residues are often absent in proteins isolated from mammalian cell cultures, even though their presence may be expected on the basis of gene sequence. Incomplete protein processing results in charge heterogeneity, which is readily identified by the ProSwift WCX-1S monolithic column. Figure 10 shows the separation of acidic, basic, and c-terminal lysine truncated variants. High loadability of the column allows purification of minor variants for further characterization.

Superior Mass Sensitivity

The mass sensitivity of the narrow bore ProSwift Ion-Exchange 1 × 50 mm columns is very high compared to the 4.6-mm i.d. column. ProSwift Ion-Exchange 1 × 50 mm columns are recommended where high sensitivity is required or sample amount is limited. Figure 11 shows that the ProSwift WAX-1S 1 × 50 mm provides excellent sensitivity.

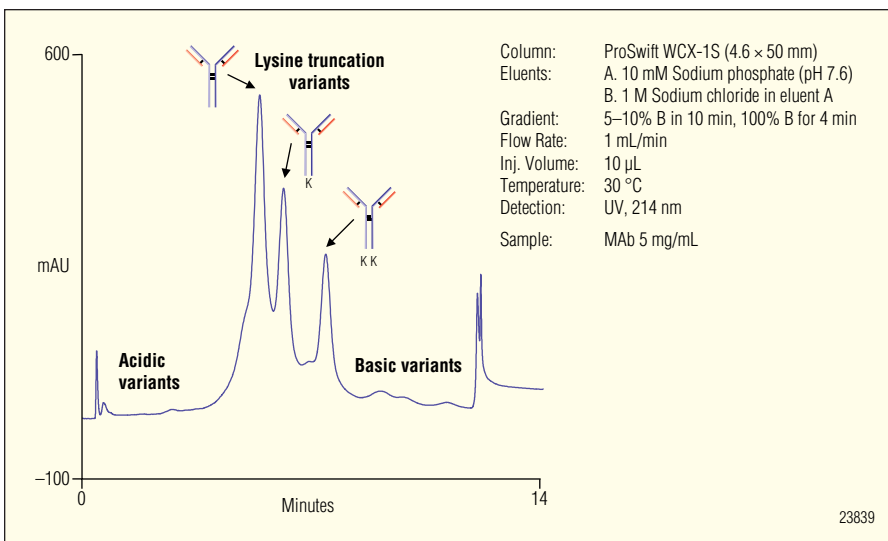


Figure 10. Separation of MAbs using the ProSwift WCX-1S monolith.

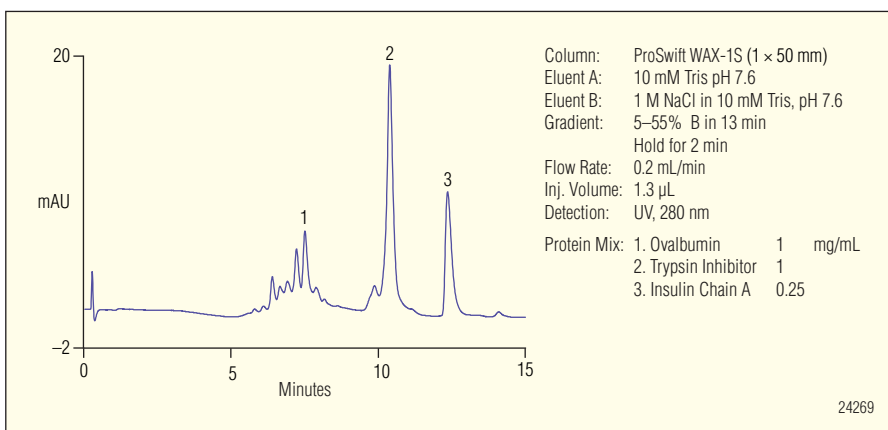


Figure 11. Mass sensitivity of the ProSwift WAX-1S monolith 1 × 50 mm.

SPECIFICATIONS

| Parameter | ProSwift WAX-1S | | ProSwift WCX-1S | | ProSwift SAX-1S | | ProSwift SCX-1S | |
|--|------------------------------|------------------------|----------------------|-----------------------|----------------------|------------------------|----------------------|------------------------|
| Column dimension | 4.6 × 50 mm | 1 × 50 mm | 4.6 × 50 mm | 1 × 50 mm | 4.6 × 50 mm | 1 × 50 mm | 4.6 × 50 mm | 1 × 50 mm |
| Base matrix material | Polymethacrylate | | | | | | | |
| Surface chemistry | Tertiary amine | | Carboxylic acid | | Quaternary amine | | Sulfonic acid | |
| Protein binding capacity per mL of polymer | 18 mg/mL BSA | 18 mg/mL BSA | 23 mg/mL Lysozyme | 23 mg/mL Lysozyme | 18 mg/mL BSA | 18 mg/mL BSA | 13 mg/mL Lysozyme | 13 mg/mL Lysozyme |
| Protein binding capacity per column | 13 mg BSA | 0.7 mg BSA | 16 mg Lysozyme | 0.9 mg Lysozyme | 13 mg BSA | 0.7 mg BSA | 9.1 mg Lysozyme | 0.5 mg Lysozyme |
| Bed height | 44 mm | 50 mm | 42 mm | 50 mm | 42 mm | 50 mm | 42 mm | 50 mm |
| Bed volume (mL) | 0.73 mL | 0.039 mL | 0.70 mL | 0.039 mL | 0.70 mL | 0.039 mL | 0.70 mL | 0.039 mL |
| pH range | 2–12 | | | | | | | |
| Recommended flow rate | 0.5–1.5 mL/min | 0.05–0.25 mL/min | 0.5–1.5 mL/min | 0.05–0.20 mL/min | 0.5–1.5 mL/min | 0.05–0.25 mL/min | 0.5–1.5 mL/min | 0.05–0.25 mL/min |
| Maximum flow rate | 2 mL/min | 0.30 mL/min | 2 mL/min | 0.25 mL/min | 2 mL/min | 0.3 mL/min | 2 mL/min | 0.3 mL/min |
| Operating pressure | < 500 psi 3.4 MPa | < 1500 psi 10.4 MPa | < 500 psi 3.4 MPa | < 1000 psi 6.8 Mpa | < 500 psi 3.4 MPa | < 1500 psi 10.4 MPa | < 500 psi 3.4 MPa | < 1500 psi 10.4 MPa |
| Maximum pressure | 1000 psi 6.9 MPa | 2000 psi 13.8 MPa | 1000 psi 6.9 Mpa | 2000 psi 13.8 Mpa | 1000 psi 6.9 MPa | 2000 psi 13.8 MPa | 1000 psi 6.9 Mpa | 2000 psi 13.8 MPa |
| Temperature | 60 °C | | | | 70 °C | | 60 °C | |
| Solvent compatibility | Most common organic solvents | | | | | | | |

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.

ProSwift Ion Exchange Monolith Columns

| | |
|--|--------|
| ProSwift Monolith WCX-1S Analytical Column (4.6 x 50 mm); PEEK™-lined stainless steel | 064295 |
| ProSwift Monolith WAX-1S Analytical Column (4.6 x 50 mm); PEEK-lined stainless steel | 064294 |
| ProSwift Monolith SAX-1S Analytical Column (4.6 x 50 mm); PEEK-lined stainless steel | 064293 |
| ProSwift Monolith SCX-1S Analytical Column (4.6 x 50 mm); PEEK-lined stainless steel | 066765 |
| ProSwift Monolith WCX-1S Analytical Column (1.0 x 50 mm); PEEK tubing | 066643 |
| ProSwift Monolith WAX-1S Analytical Column (1.0 x 50 mm); PEEK tubing | 066642 |
| ProSwift Monolith SAX-1S Analytical Column (1.0 x 50 mm); PEEK tubing | 068459 |
| ProSwift Monolith SCX-1S Analytical Column (1.0 x 50 mm); PEEK tubing | 071977 |

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