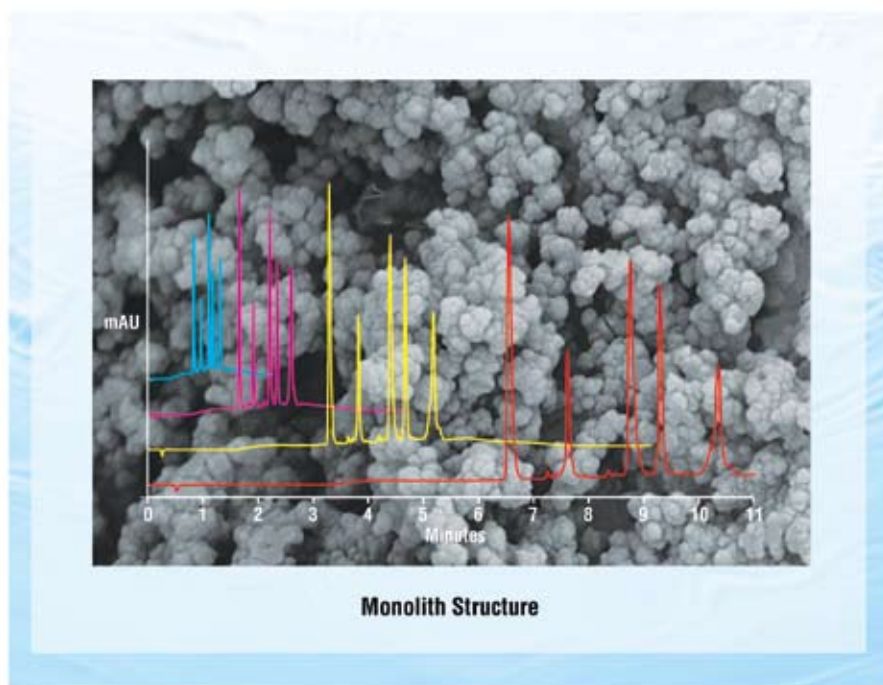


columns

ProSwift Reversed-Phase Monolith Columns for Protein Analysis



ProSwift® reversed-phase columns use a unique monolith technology for fast, high-resolution HPLC and LC/MS separations of proteins. These monolith columns have several advantages over traditional packed columns, including the outstanding resolving power of nonporous media combined with faster separations than bead-based columns. Low backpressures and fast mass transfer characteristics allow the use of high flow rates for faster chromatographic separations with minimal loss of resolution. In addition, ProSwift reversed-phase columns have exceptional reproducibility, pH stability, and high stringent wash compatibility, providing a longer column lifetime than silica-based columns.

Major Column Features and Benefits

ProSwift reversed-phase (RP) monoliths are a family of high-resolution columns for protein analysis. Their unique monolith design provides:

- Fast mass transfer
- Fast separations
- High throughput and improved productivity
- Long column lifetime
- Outstanding reproducibility
- Low carryover

Standard HPLC Columns

ProSwift RP columns for standard HPLC systems include the ProSwift RP-1S, RP-2H, RP-3U, and RP-4H. Additional benefits include:

- High flow rates
- Low backpressures
- Excellent stability over a wide pH range
- High stringent wash compatibility

Micro HPLC Columns

The ProSwift RP-10R column for micro HPLC systems is the ideal choice for fast, very high-resolution protein applications.



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Porous Polymeric Monoliths

The unique monolith support of ProSwift RP columns 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, while delivering low backpressures that support retained resolution at increased linear velocities. Therefore, the morphology of the monolith allows protein separations to be run at high flow rates while maintaining high resolution.

Fast Separations for High Throughput

The high resolution and high-flow-rate qualities of the ProSwift columns provide for fast separation through the use of steep gradients and high linear velocities. Figure 1 shows the ability of the Pro-Swift RP-2H to separate protein mixtures at a wide range of flow rates. Increasing the flow rate to 8 mL/min decreases the sample separation time to less than 2 min while still providing high resolution. This fast separation demonstrates the exceptionally high-throughput capabilities available with ProSwift columns. The resulting decrease in analysis time translates into a five- to ten-fold increase in productivity as compared to conventional bead-based columns. For example, if only three samples per hour can be evaluated using a conventional column at 1 mL/min, up to 24 samples can be evaluated per hour using a ProSwift column at 8 mL/min while maintaining relatively high resolution. This benefit is especially significant for those laboratories requiring high throughput screening capabilities such as fast QA/QC process monitoring. Through the use of steep gradients on the ProSwift RP columns, high throughput screening needs can be met (Figure 2).

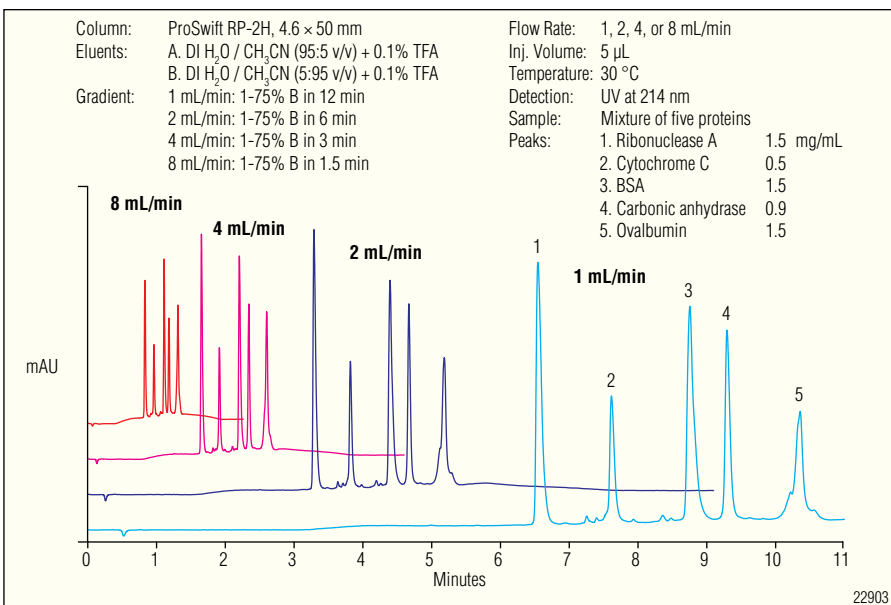


Figure 1. Fast, high-resolution separation of proteins, run on a standard HPLC system.

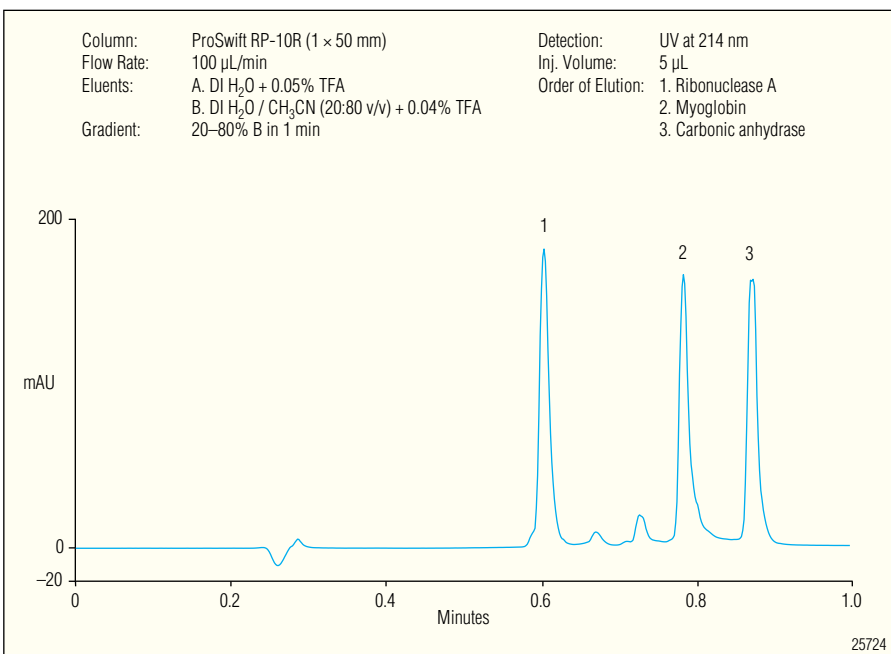


Figure 2. Fast separation with the use of a steep gradient on the ProSwift RP-10R column, run on a micro HPLC system.

High Resolution

ProSwift RP columns are high-resolution columns that have minimal loss of resolution even at high linear velocities. At increased linear velocities, the high resolution of ProSwift RP monolith columns is achieved by a fast mass transfer that is primarily driven by convective flow through the large channels and nonporous surfaces of the monolith. Mass transfer of large biomolecules such as proteins in and out of pores of conventional porous-bead packing material is limited by low diffusivity and steric hindrance, resulting in peak broadening and poor resolution, especially at high flow rates. ProSwift RP columns minimize these problems. For example, Figure 3 shows a comparison between a ProSwift RP-2H monolith column and a porous-bead column. The ProSwift RP-2H column demonstrates higher resolution and faster separations at the same flow rate as a competitor's column, and high resolution at an increased flow rate that the competitor column can not achieve. The ProSwift RP-4H can be run over a wide range of flow rates from 0.1 mL/min to 0.5 mL/min while maintaining resolution (Figure 4). These MS compatible flow rates also consume less solvent.

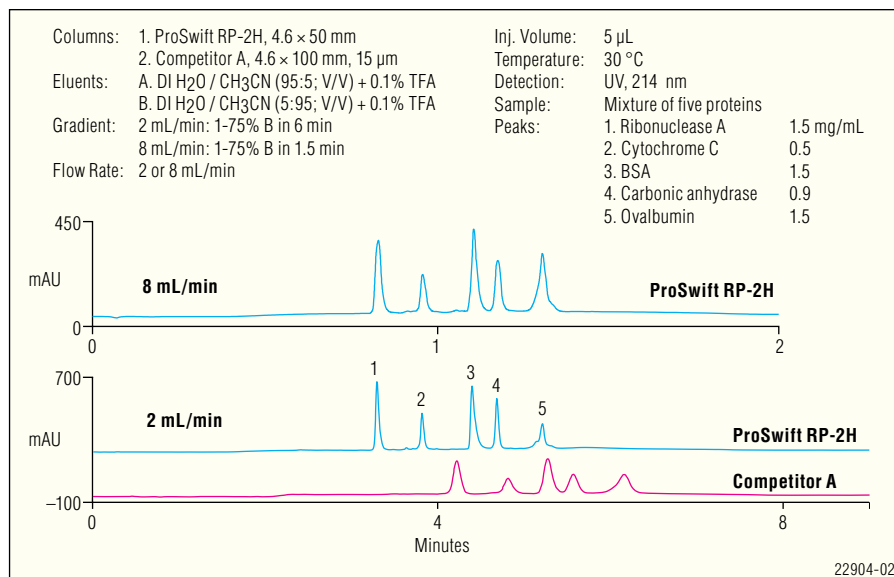


Figure 3. Comparison of ProSwift monolith with porous HPLC media. Gradients, concentrations of analytes, and system are the same as in Figure 1.

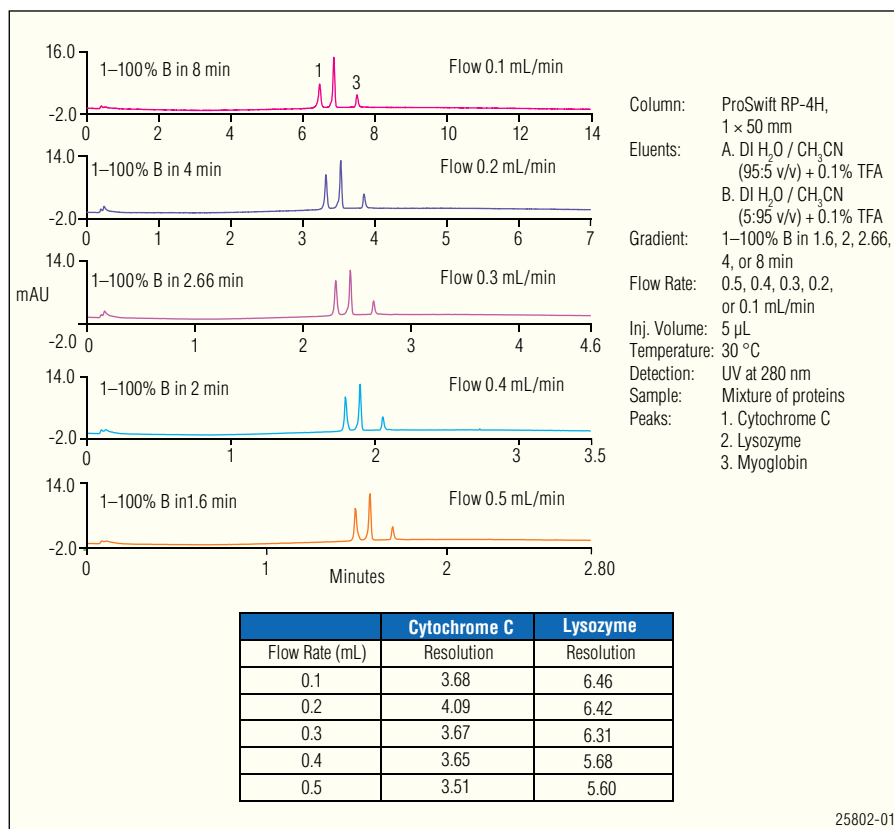


Figure 4. Minimal loss of resolution at increased flow rates using the ProSwift RP-4H column. Experiments were performed using a standard HPLC system.

Column Diversity

The ProSwift RP columns are available in various porosities, linear velocity ranges, and formats. These high-resolution columns, each having a wide linear velocity range, are capable of separating protein samples of various complexities and hydrophobicities. The porosities include standard (RP-1S), high (RP-2H and RP-4H), and ultrahigh (RP-3U) for use on standard HPLC systems. The reduced porosity of the RP-10R is specifically designed for use with micro HPLC systems. This range of columns provides for easy optimization, excellent performance, and high-throughput capability. Figure 5 illustrates how the differences in morphology of the 4.6 mm columns effect the separation. The small pore sizes of the ProSwift RP-1S and RP-10R columns provide the highest efficiency and resolution (4.6×50 and 1×50 mm, respectively) while the ProSwift RP-2H and RP-4H (4.6×50 and 1×50 mm, respectively) are ideal for high throughput applications and screening.

Column Length

The ProSwift RP-1S, RP-2H, RP-3U, RP-4H and the RP-10R are all available in a 50 mm length. This short length of the column allows for fast separations and increased sample throughput. The ProSwift RP-4H is also available in a 250 mm length, to provide increased peak capacity while still allowing fast separations. Figure 6 shows the excellent resolution of the ProSwift RP-4H 1×250 mm column and the shorter run time of the ProSwift RP-H 1×50 mm.

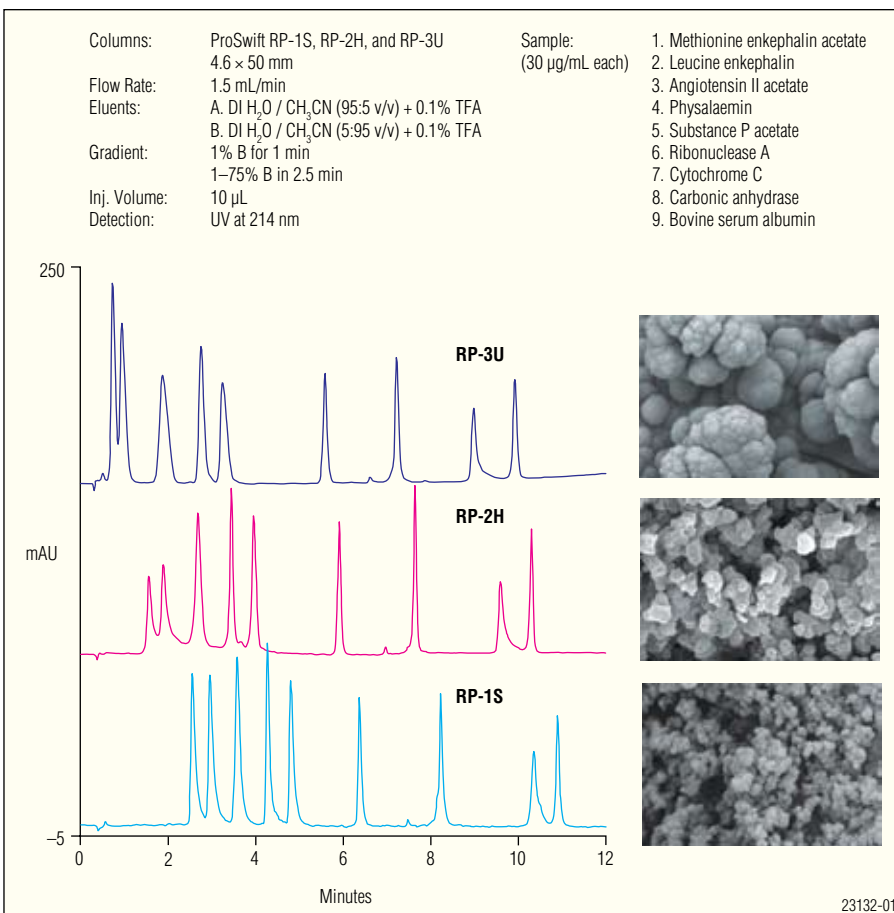


Figure 5. Separation of proteins and large peptides on ProSwift RP 4.6 mm i.d. columns, run using a standard HPLC system. The SEM photos show the morphologies of the different columns.

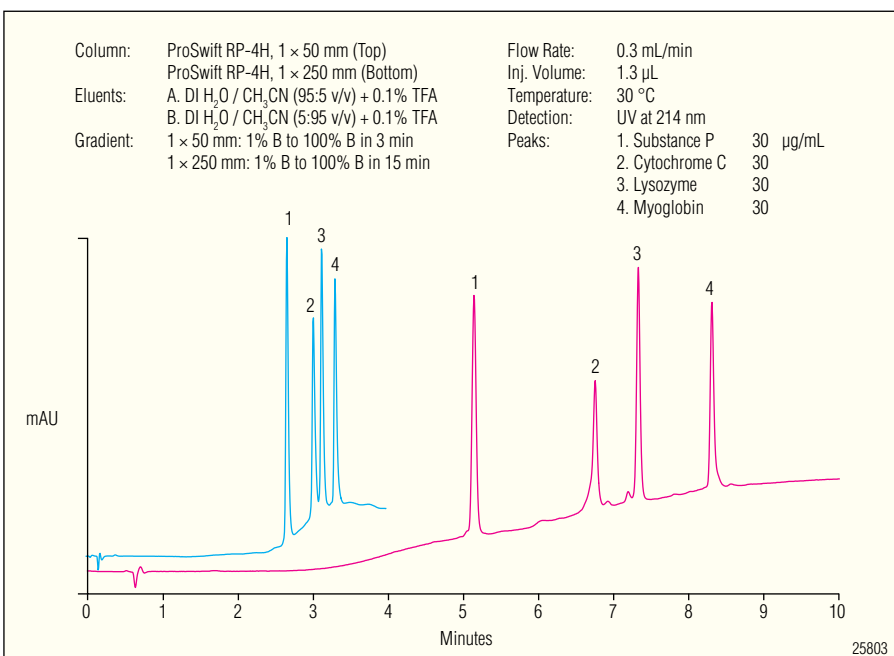


Figure 6. The ProSwift RP-4H 1×50 mm column enables fast separations (top chromatogram) while the 1×250 mm column provides high resolution and increased peak capacity (bottom chromatogram). Experiments were performed using a standard HPLC system.

Column Diameter

The ProSwift reversed-phase columns are available in 4.6 and 1.0 mm inner diameter formats. The 4.6 mm format is ideal for common analytical applications while the 1.0 mm format provides the advantages of increased mass sensitivity and lower sample and solvent consumption, making it ideal for LC/MS applications. For example, Figure 7 shows the increased mass sensitivity of the 1.0 mm RP-4H column (chromatogram B) over the 4.6 mm RP-2H column (chromatogram A). Figure 8 demonstrates the 2-D LC separation of *E. coli* proteins in which the ProSwift RP-10R 1 × 50 mm column was used as the second-dimension column. The RP-10R column provides fast, high resolution separations of complex protein samples, ideal for top-down protein mass spectrometric analysis.

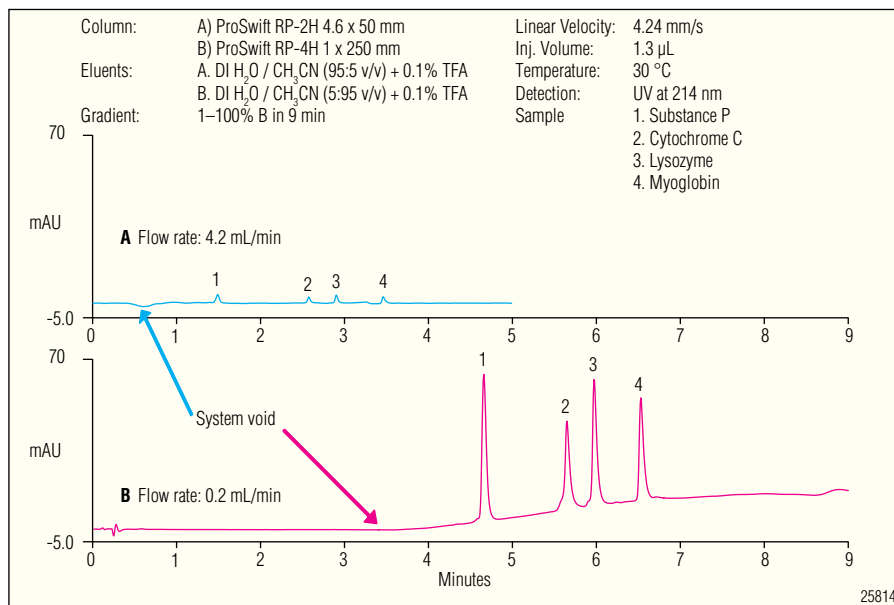


Figure 7. Comparison of mass sensitivity for a protein separation on a 4.6 × 50 mm ProSwift RP-2H column (chromatogram A) and a 1 × 250 mm ProSwift RP-4H column (chromatogram B), both using a standard HPLC system. The smaller diameter column yields a much higher signal, allowing lower detection limits.

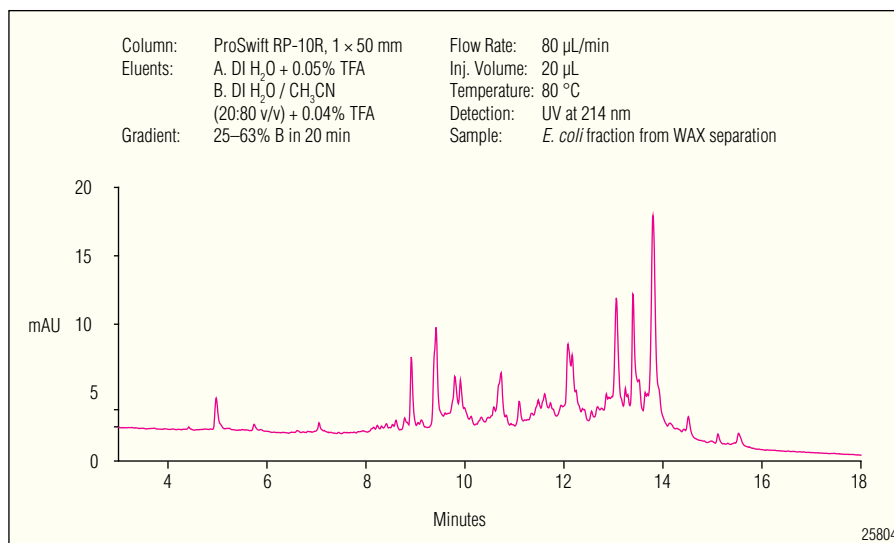


Figure 8. 2-D LC separation of a complex intact protein mixture (*E. coli*). First-dimension ion-exchange separation was carried out on a 4.6 × 50 mm ProSwift WAX-1S column (not shown). The second-dimension reversed-phase separation of collected fraction nine was carried out on a 1 × 50 mm ProSwift RP-10R column (shown above). Experiments were performed using a micro HPLC system.

Column Reproducibility and Ruggedness

The ProSwift RP columns, with their outstanding reproducibility, provide longer lifetimes than bead- and silica-based columns. For example, Figures 9 and 10 demonstrate the long column lifetimes of the ProSwift RP-1S and RP-4H columns, respectively. These overlays of representative chromatograms of multiple injections demonstrate that column reproducibility and high resolution are maintained over hundreds of runs. This feature is consistent with all ProSwift columns.

The ProSwift RP columns designed for standard systems have a broad pH range of 1–14, providing high-stringency washing capabilities (1M NaOH) for sterilization or removal of precipitated proteins. Silica columns have a limited pH range (typically to pH 7.5), which does not allow for high stringency washing, thus limiting their lifetime. ProSwift RP columns also have lower carryover than bead-based columns.

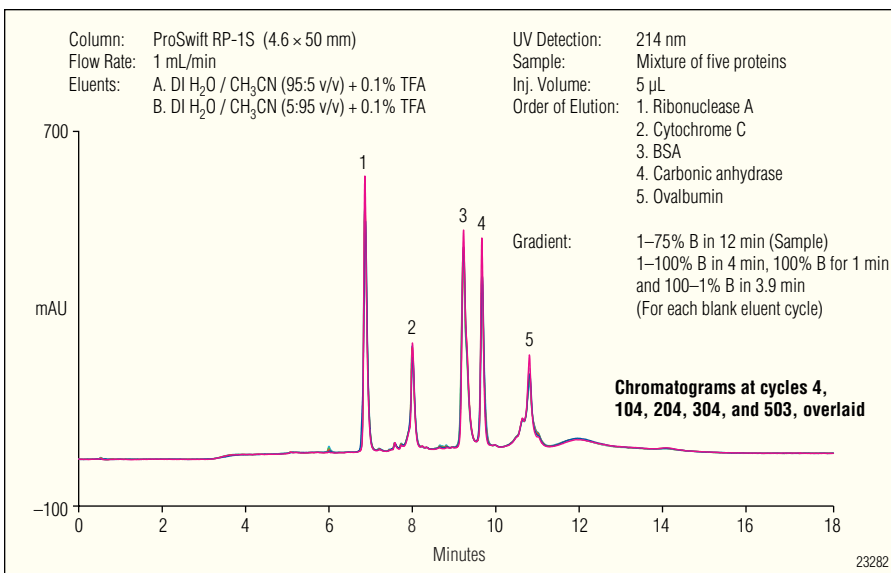


Figure 9. Reproducibility testing of the ProSwift RP-1S column. Concentrations of analytes and system used are the same as in Figure 1.

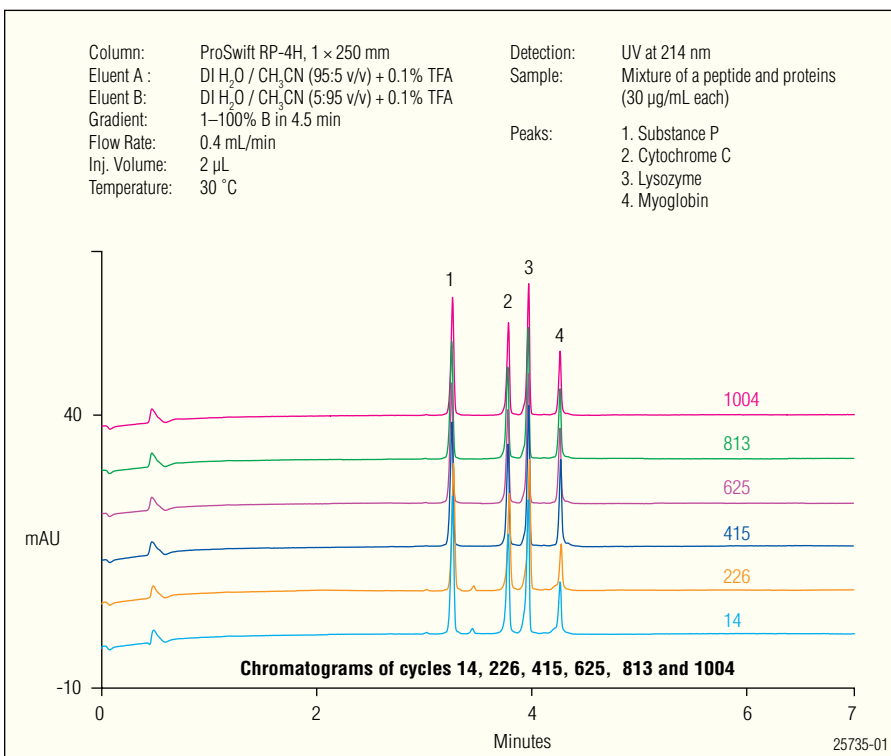


Figure 10. Reproducibility testing of the ProSwift RP-4H column, performed using a standard HPLC system.

SPECIFICATIONS

Parameter	ProSwift RP-1S	ProSwift RP-2H	ProSwift RP-3U	ProSwift RP-4H		ProSwift RP-10R
Column dimension	4.6 × 50 mm	4.6 × 50 mm	4.6 × 50 mm	1 × 50 mm	1 × 250 mm	1 × 50 mm
Base matrix material	Polystyrene-divinylbenzene					
Surface chemistry	Phenyl					
Protein binding capacity per mL of polymer	3.0 mg/mL Lysozyme	1.0 mg/mL Lysozyme	0.5 mg/mL Lysozyme	2.3 mg/mL Lysozyme		n.d.*
Protein binding capacity per column	2.2 mg Lysozyme	0.8 mg Lysozyme	0.3 mg Lysozyme	0.1 mg Lysozyme	0.5 mg Lysozyme	n.d.
Bed height	45 mm	43 mm	40 mm	50 mm	250 mm	50 mm
Bed volume (mL)	0.76 mL	0.73 mL	0.65 mL	0.039 mL	0.196mL	0.039 mL
pH range	1–14					1–10
Recommended flow rate	0.5–4 mL/min	1–10 mL/min	1–16 mL/min	0.1–0.4 mL/min	0.1–0.3 mL/min	40–60 µL/min
Maximum flow rate	5 mL/min	14 mL/min	20 mL/min	0.5 mL/min	0.4 mL/min	100 µL/min
Operating pressure	< 1500 psi 10.4 MPa				< 2500 psi 17.2 MPa	2900 psi 20 MPa
Maximum pressure	2800 psi 19.3 MPa			2000 psi 13.8 Mpa	3000 psi 20.7 MPa	4350 psi 30 MPa
Temperature	70 °C					80 °C
Solvent compatibility	Most common organic solvents					

*n.d.= not determined

KEY BENEFITS OF PROSWIFT RP MONOLITH COLUMNS

Benefit	4.6 mm i.d. Columns			1 mm i.d. Columns	
	RP-1S	RP-2H	RP-3U	RP-4H	RP-10R
High Resolution	X	X		X	X*
Fast Mass Transfer	X	X	X	X	X
Fast Separations	X	X	X	X	X
High Throughput/ Improved Productivity	X	X	X	X	X
Long Column Lifetime	X	X	X	X	X
Outstanding Reproducibility	X	X	X	X	X
Low Carryover	X	X	X	X	X
High Linear Velocities	X	X	X	X	
Low Backpressures	X	X	X	X	
Excellent pH Stability	X	X	X	X	
High Stringent Wash Compatibility	X	X	X	X	
High Mass Sensitivity				X	X
Small Proteins	X	X		X	X
Large Proteins	X	X	X	X	X
Standard HPLC	X	X	X	X	
Micro HPLC				X	X

*For 1 mm i.d. columns, RP-10 is recommended for highest 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.

ProSwift Reversed-Phase Monolith Columns	Part Number
ProSwift Monolith RP-1S, 4.6 × 50 mm; stainless steel.....	064297
ProSwift Monolith RP-2H, 4.6 × 50 mm; stainless steel	064296
ProSwift Monolith RP-3U, 4.6 × 50 mm; stainless steel.....	064298
ProSwift Monolith RP-4H, 1 × 50 mm; PEEK™	069477
ProSwift Monolith RP-4H, 1 × 250 mm; PEEK.....	066640
ProSwift Monolith RP-10R, 1 × 50 mm; glass-lined stainless steel.....	164397

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Dionex Corporation

1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

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