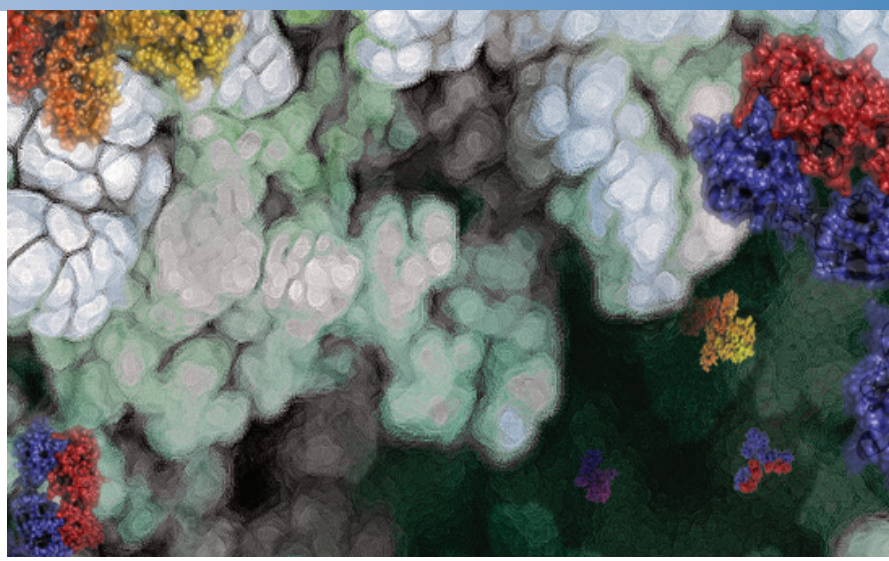
PepSwift and ProSwift

Monolithic capillary columns

Thermo Scientific™ PepSwift™ and ProSwift™ monolithic columns are specially designed for high-resolution LC-MS analysis in protein identification, biomarker discovery, and systems biology. Based on a continuous, porous copolymer bed, the monolithic structure offers a high-quality alternative to traditional microparticulate sorbents, providing important advantages to the chromatographic separation. High-sensitivity proteomics and biotech applications are easily performed using these columns.

- High-resolution columns for protein identification, biomarker discovery, and systems biology
- Designed to enable rapid peptide and protein separations
- Wide range of dimensions available for nano and capillary LC-MS applications
- Superior lifetime
- Thermo Scientific™ nanoViper™ fittings for easy column installation



Example Applications

Peptides	Low–Medium MW Proteins (≤30 kDa)	High MW Proteins (>30 kDa)
• Peptide-based drugs • Tryptic digests • Bottom-Up Proteomics	• Partial protein digests • Low MW protein drugs	• Monoclonal Antibodies • Cell Lysates • Top-Down Proteomics

Column Formats

Columns are available in different formats and chemistries designed to obtain the best column performance.

For ≤200 µm these include PEEK housing with nanoViper fittings. For 500 µm these include PEEK housing with standard PEEK end fittings to allow connection to nanoViper tubing. All columns are made of biocompatible materials.

PepSwift and ProSwift columns are available in a range of formats that operate at flow rates compatible with nano, LC-MS for the detection of peptides and proteins. Low nanoliter and microliter flow rates used with the 50 µm and 100 µm i.d. columns are well suited for top-down and bottom-up research proteomics applications where separation of a large number of proteins is desirable using a high resolution mass spectrometer from a low volume sample.

The 50 µm i.d. ProSwift C4 RP-5H provides increased sensitivity for clinical research applications.

The flow rates used with 500 µm i.d. columns enable fast separations for high throughput analysis of multiple samples necessary to identify target proteins for tissue or bacterial specie identification. The 200 µm i.d. columns bridge the gap between the two other formats enabling higher throughput while providing excellent resolution.

For ProSwift RP-4H and PepSwift columns, the PepSwift monolithic trap columns can be used for preconcentration and desalting of samples consisting of peptides and proteins. The sample capacity of the PepSwift precolumns (200 µm i.d. × 5 mm) is in the range of 100 pmol for both peptides and proteins.

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Column Chemistry

Monolith capillary columns are available in a range of different chemistries: PepSwift phases possess phenyl functionality based on a poly(divinylbenzene-co-ethylvinylbenzene-styrene) co-polymer. ProSwift RP-4H phases are based on a poly(divinylbenzene-co-ethylvinylbenzene) co-polymer with similar selectivity to the PepSwift phase. The ProSwift C4 RP-5H phase possesses butyl functionality based on a poly(ethylene dimethacrylate-co-butyl methacrylate) co-polymer providing a less hydrophobic selectivity.

Backpressures and Pore Size Distributions

The PepSwift and the ProSwift RP-4H monolith columns have a smaller modal pore size for increased separation efficiency. The increased surface area of the PepSwift monolith makes this the best choice for peptides, smaller proteins and protein fragments when operating at higher temperatures and pressures. The ProSwift columns have a larger pore volume therefore operate at lower backpressures than the PepSwift columns, providing excellent efficiency at room temperature for intact proteins and fragments.

Resolution and Speed of Separation

The uniquely designed morphology of PepSwift and ProSwift monoliths allows fast analyte mass transfer minimizing band broadening at increased flow rates. This allows faster separations with less loss of resolution compared to typical porous bead based media. This is especially true for large molecules whose diffusivities are much lower than small molecules.

The dominance of convective mass transfer can be taken advantage of at high flow rates. By increasing the flow rate, using the same gradient, faster separations can be achieved with increased peak capacity.

PepSwift and ProSwift monolith columns offer excellent separation at low and high flow rates which improves operational versatility and productivity.

At elevated temperatures the diffusivity of peptides increases resulting in faster transport to the stationary phase to provide increased interactions with the PepSwift monolith. Operating at 60 °C, the PepSwift column can separate a peptide mixture with peak widths at half height of only 1.6–3.5 seconds and base line resolution of each peptide using only a 7 minute gradient.

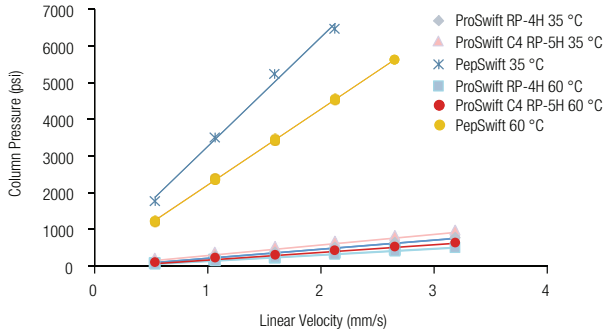


Figure 1: Column backpressure as a function of eluent flow rate at 35 °C and 60 °C for a ProSwift RP-4H, a ProSwift C4 RP-5H column, and a PepSwift column. All column formats are 100 µm × 25 cm, eluent: 90.5/9.5 water/acetonitrile + 0.1% trifluoroacetic acid

Column:	**ProSwift RP-4H** 200 µm × 25 cm	Sample:	Peptides and proteins each at 5 µg/mL in DI water + 0.1% trifluoroacetic acid
Eluents:	A: water/acetonitrile (95/5 v/v) + 0.1% trifluoroacetic acid B: water/acetonitrile (5/95 v/v) + 0.1% trifluoroacetic acid	Peaks:	1. Angiotensin II 2. Substance P 3. Lysozyme 4. Myoglobin 5. L-Lactic Dehydrogenase
Gradient*:	1–65% B in 10 minutes		
Flow Rate:	2, 4, and 8 µL/min		
Inj. Volume:	1 µL		
Temp.:	35 °C	*5 minute pre-run equilibration	

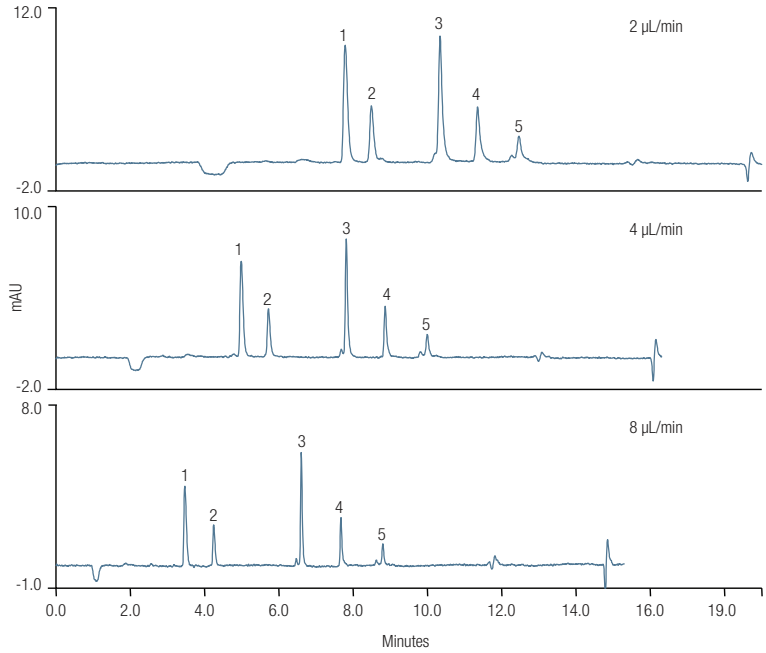
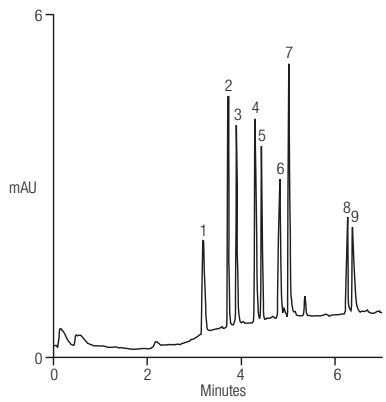


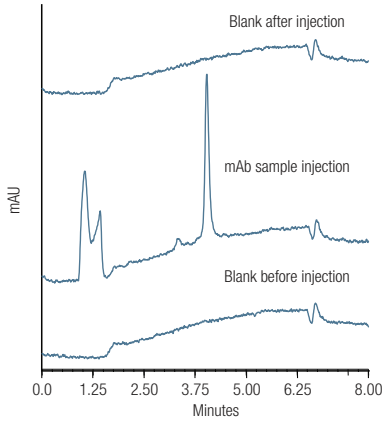
Figure 2: The effect of increased flow rate using the same gradients on the ProSwift RP-4H, 200 µm × 25 cm monolith column



Peaks:
1. Bradykinin fragments 1–5
2. Vasopressin [Arg8]
3. Methionine enkephalin
4. Leucine enkephalin
5. Oxytocin
6. Bradykinin
7. LHRH
8 Bombesin
9. Substance P

Injected amount: 500 fmol each
Detection: UV 214 nm, response time 0.1 sec.

Figure 3: Separation of a 9 peptide mixture on a PepSwift column at 60 °C



Column:	**ProSwift C4 RP-5H** 200 µm × 25 cm
Eluents:	A: water + 0.05% trifluoroacetic acid B: water/acetonitrile (20/80 v/v) + 0.04% trifluoroacetic acid
Gradient*:	5–70% B in 5 minutes
Flow Rate:	8 µL/min
Inj. Volume:	1 µL
Temp.:	35 °C
Detection:	UV at 214 nm
Sample:	Monoclonal antibody – 10 µg/mL in DI water + 0.05% trifluoroacetic acid

*5 minute pre-run equilibration

Figure 4: Analysis of a monoclonal antibody on a 200 µm x 25 cm ProSwift C4 RP-5H column demonstrating no protein carryover between runs

Column:	**PepSwift Monolithic Capillary** 200 µm i.d. × 50 mm
Eluents:	A. DI water + 0.05% TFA B. DI water/acetonitrile (50:50 v/v) + 0.04% trifluoroacetic acid
Gradient:	0–70% B in 15 min, 90% B for 5 min 10 min equilibration
Flow Rate:	2.5 µL/min
Inj. Volume:	200 nL
Temp.:	60 °C
Detection:	UV at 214 nm ESI-MS/MS, positive ion mode, mass range 300–1600 *m/z*
Sample:	Protein mix digest (cytochrome C, lysozyme, alcohol dehydrogenase, bovine serum albumin, apo-transferrin, beta-galactosidase, 100 fmol each)

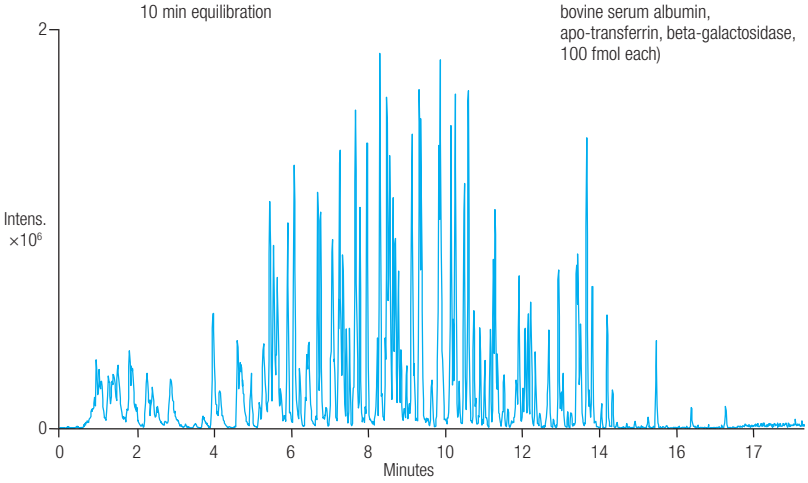


Figure 5: MS chromatogram of a capillary LC separation of over 100 peptide fragments, obtained in less than 15 minutes using a PepSwift column.

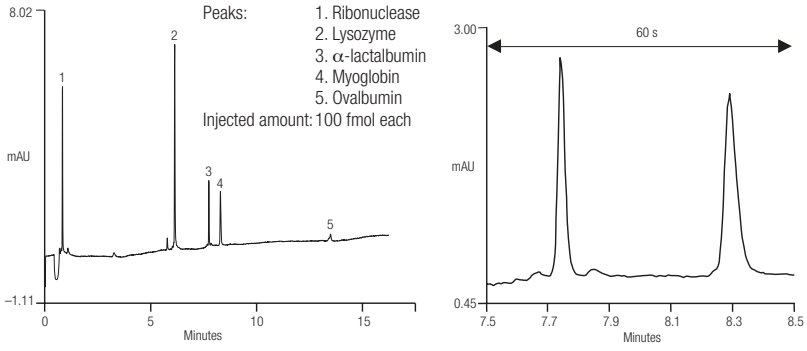


Figure 6: (Left) Separation of 5 proteins using a 200 µm ID PepSwift column, and 1. Ribonuclease A (14 kDa), 2. Lysozyme (14.3 kDa), 3. α-Lactalbumin (25.6 kDa), 4. Myoglobin (17.1 kDa), 5. Ovalbumin (44.3 kDa); and (right) an enlarged region of the chromatogram showing the symmetrical shape and narrow peak widths of peaks 3 and 4

Low Carryover

Carryover can be problematic especially in the analysis of antibodies and antibody-drug conjugates. The molecular weight of antibodies is generally on the order of ~150 kDa. Proteins of this size can often bind strongly to the column and cause carryover from injection to injection. As shown in Figure 4, ProSwift C4 RP-5H monolith columns are capable of separating antibodies without significant carryover.

High-Speed, High-Resolution LC-MS/MS Peptide Mapping

PepSwift capillary monolithic columns are an ideal choice for peptide sequencing and excellent separation efficiencies are routinely obtained in LC-MS applications. Figure 5 shows the MS base peak chromatogram of a fast and highly efficient LC-MS separation of six digested proteins.

Low Molecular Weight Protein Samples

Many low molecular weight proteins can be separated using PepSwift columns. In general, filtered protein samples below a molecular weight of ~30 kDa can be separated without compromising the performance of the column due to fouling. Applications include column or procedure validation, QA/QC, investigation of low molecular weight protein-based drugs, and top-down proteomics analysis of low-molecular weight protein fractions. Figure 6 shows the separation of a simple protein set of 4 low molecular weight proteins and one high molecular weight protein on a 200 µm ID PepSwift column using a gradient of 20–50% acetonitrile in water and 0.05% trifluoroacetic acid over 15 minutes.

High Molecular Weight Proteins

Cell Lysates and GELFrEE Fractions

The analysis of cell lysates and corresponding GELFrEE fractions is extremely important in the areas of top-down proteomics and clinical research of healthy and diseased tissues. These samples are very complex, containing thousands of proteins and other material from the cell lysate. The high loading capacity and separation capabilities of the ProSwift RP-4H and ProSwift C4 RP-5H columns enables the separation and MS detection of a large number of proteins when analyzing these complex samples.

Figure 7 shows the separation of GELFrEE fractions from human HeLa cells (MW range: ~11–23 kDa and 20–52 kDa) using a 100 µm × 50 cm ProSwift RP-4H column with MS detection. The ability to separate a large number of proteins in both low and high molecular weight samples makes the ProSwift RP-4H an ideal choice for these types of samples.

Monoclonal Antibodies

The primary structure of monoclonal antibodies (mAbs) can have a great impact upon their efficacy as biotherapeutic drugs. It is important that the structure is fully characterized during design and development.

Coupled with mass spectrometry, monolithic capillary columns can be used to characterize glycosylation and other post-translational modifications of the mAb as well as digests and fragmentation patterns.

Structural variants can often be observed as minor peaks before or after the main mAb peak depending on whether the variant is less or more hydrophobic than the main mAb structure, respectively. Figure 8 demonstrates the separation of multiple minor variants from the main mAb structure.

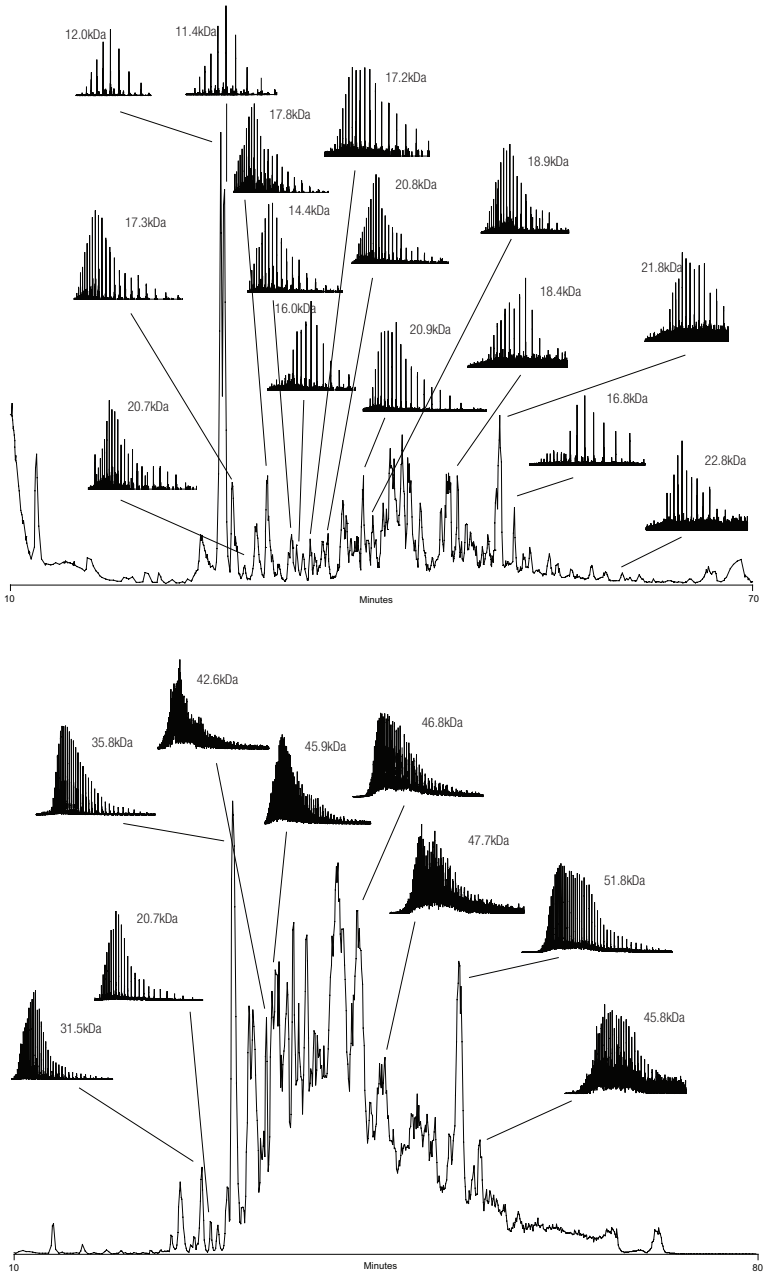


Figure 7: Separation of GELFrEE fractions from HeLa cell lysates on a 100 µm × 50 cm ProSwift RP-4H column using mass spectrometry for protein detection and identification. Data courtesy of Prof. Neil Kelleher, Northwestern University, Evanston, IL

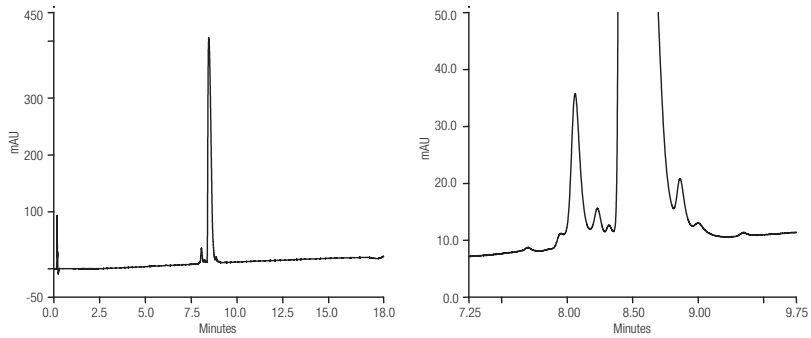


Figure 8: Separation of mAb variants using a 500 µm x 10 cm ProSwift C4 RP-5H column. The separation was performed at a temperature of 85 °C and a flow rate of 150 µL/min using a gradient from 12–55% B over 15 minutes with 97.5/2.5 (v/v) water/acetonitrile + 0.1% trifluoroacetic acid for eluent A and 10/90 water/acetonitrile + 0.08% trifluoroacetic acid for eluent B.

PepSwift and ProSwift Monolithic Capillary Column Specifications

Column	PepSwift and ProSwift RP-4H	ProSwift C4 RP-5H
Surface Chemistry	Phenyl	Butyl
pH Range	1–10	
Maximum Temperature*	90 °C	
Solvent Compatibility	Most common organic solvents (Refer to column manual)	

*For EASY-Spray maximum operating temperature, please refer to EASY-Spray product brochure for specifications.

Operational Parameters

Column	Column Dimension	Recommended Flow Rate Range, µL/min	Maximum Pressure, psi (Mpa)	Dynamic Binding Capacity per mL of Monolith
PepSwift	100 µm × 5 cm	0.7–1.0	4350 (30.0)	1.6 µg/mL tryptic cytochrome C digest
	100 µm × 25 cm			
	200 µm × 5 cm	2–3		
	200 µm × 25 cm			
	500 µm × 5 cm	15–25		
ProSwift RP-4H	100 µm × 50 cm	0.5–3.0	4950 (34.1)	59.9 µg/mL α-Chymotrypsinogen
	100 µm × 25 cm	0.5–3.0		
	200 µm × 25 cm	2–12		
	500 µm × 10 cm	12–80	2000 (13.8)	
ProSwift C4 RP-5H	50 µm × 25 cm	0.25–0.75	4950 (34.1)	40.0 µg/mL α-Chymotrypsinogen
	100 µm × 50 cm	0.5–3.0		
	100 µm × 25 cm			
	200 µm × 25 cm	2–12		
	500 µm × 10 cm	12–100	2000 (13.8)	
	500 µm × 25 cm			

Typical operating pressures at 35 °C for ProSwift RP-4H and ProSwift C4 RP-5H columns: 100 µm × 50 cm: <3300 psi @ 2 µL/min; 100 µm × 25 cm: <1650 psi @ 2 µL/min; 200 µm × 25 cm: <1650 psi @ 8 µL/min; 500 µm × 10 cm < 660 psi @ 50 µL/min; ProSwift C4 RP-5H 50 µm × 25 cm: <1650 psi @ 0.5 µL/min; 500 µm × 25 cm: <1650 psi @ 50 µL/min. Further information is provided in the column manuals.

Ordering Information

PepSwift Monolith Columns	Part Number
PepSwift Monolithic Nano Column, 100 µm ID × 5 cm, nanoViper	164584
PepSwift Monolithic Nano Column, 100 µm ID × 25 cm, nanoViper	164543
PepSwift Monolithic Capillary Column, 200 µm ID × 5 cm, nanoViper	164557
PepSwift Monolithic Capillary Column, 200 µm ID × 25 cm, nanoViper	164542
PepSwift Monolithic Capillary Column, 500 µm ID × 5 cm, nanoViper	164585
EASY-Spray PepSwift Monolithic Capillary Column, 200 µm ID × 25 cm, nanoViper	ES810

PepSwift Trap Columns	Part Number
PepSwift Monolithic Trap Column, 200 µm × 5 mm, (set of 2), nanoViper	164558

ProSwift RP-4H Monolith Columns	Part Number
ProSwift RP-4H Monolithic Nano Column, 100 µm ID × 50 cm, nanoViper	164921
ProSwift RP-4H Monolithic Nano Column, 100 µm ID × 25 cm, nanoViper	164922
ProSwift RP-4H Monolithic Capillary Column, 200 µm ID × 25 cm, nanoViper	164923
ProSwift RP-4H Monolithic Capillary Column, 500 µm ID × 10 cm, 10–32	164925

ProSwift C4 RP-5H Monolith Columns	Part Number
ProSwift C4 RP-5H Monolithic Nano Column, 50 µm ID × 25 cm, nanoViper	164935
ProSwift C4 RP-5H Monolithic Nano Column, 100 µm ID × 50 cm, nanoViper	164928
ProSwift C4 RP-5H Monolithic Nano Column, 100 µm ID × 25 cm, nanoViper	164929
ProSwift C4 RP-5H Monolithic Nano Column, 200 µm ID × 25 cm, nanoViper	164930
ProSwift C4 RP-5H Monolithic Nano Column, 500 µm ID × 10 cm, 10–32	164931
ProSwift C4 RP-5H Monolithic Nano Column, 500 µm ID × 25 cm, 10–32	164932

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