

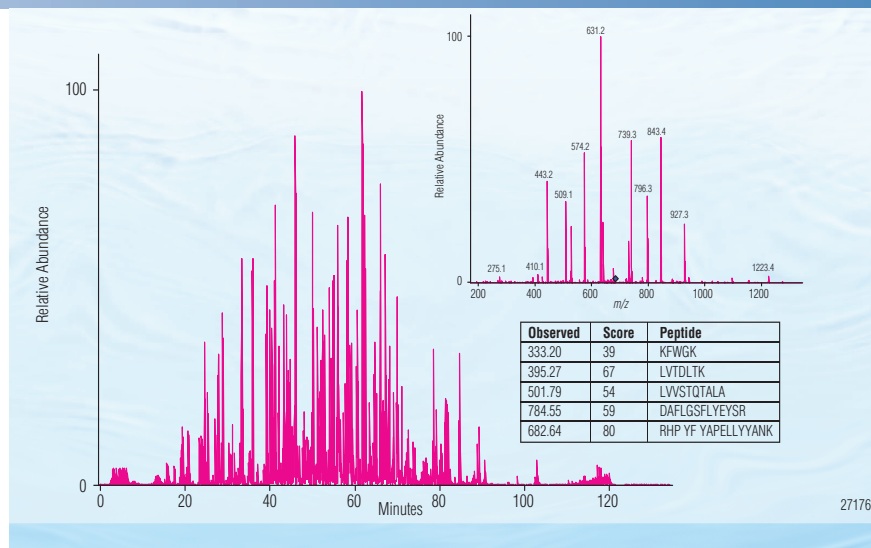
Acclaim PepMap and Acclaim PepMap RSLC Columns

for High-Resolution Peptide Mapping

Thermo Scientific™ Acclaim™ PepMap™ and Acclaim PepMap RSLC columns are specially designed for high-resolution analyses of tryptic, natural, and synthetic peptides. The Acclaim PepMap columns are often applied for LC-MS/MS peptide mapping for protein identification, biomarker discovery, and systems biology. Due to their high loading capacity, the Acclaim PepMap columns are exceptionally suitable for the analysis of low abundant peptides in complex proteomics samples.

Column Features

- High resolution in protein identification, biomarker discovery, and systems biology
- Highest sensitivity in LC/MS due to unique loadability
- Designed for TFA-free LC-MS, minimizing ion-suppression effects
- Ideally suited for coupling to ESI/MS and MALDI-MS
- Highest column-to-column reproducibility
- Easy-to-use, cutting-edge miniaturized HPLC
- Thermo Scientific™ nanoViper™ fingertight connection system for easy column installation



High-Resolution Peptide Mapping

Acclaim PepMap LC columns are uniquely suited for the analysis of complex peptide samples, and are available in different column diameters and lengths. As sample amounts continue to become smaller, the reduction of column diameters is the answer to obtain the highest detection sensitivity.

The 75 μm i.d. nano LC columns, (available in 5, 15, 25, and 50 cm formats) deliver fast high-resolution LC-MS separations. Due to unique bonding chemistry, the Acclaim PepMap columns exhibit an exceptionally high loading capacity. This is a very important asset when analyzing low abundant peptides in complex proteomics samples.

Acclaim PepMap RSLC Columns for Ultrahigh Resolution Separations

The Acclaim PepMap stationary phase has become the standard for peptide separations in proteomics and can be used with all modern nano LC systems available in the market. Building on this success, the Acclaim PepMap RSLC stationary phase has been developed for ultra-high resolution peptide separations. The 2 μm particles of this stationary phase yield smaller peak widths.

A high pressure nano LC system, such as the Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLCnano system with an 800 bar pressure rating, allows full utilization of the potential of the Acclaim PepMap RSLC columns. An example of this excellent performance is demonstrated in Figure 1 with the ultra-high resolution LC-MS separation of a complex *E. coli* tryptic digest sample.

Nomenclature	Column i.d.	Typical Flow Rate
Micro LC	1 mm	40 $\mu\text{L}/\text{min}$
Capillary LC	300 μm	4 $\mu\text{L}/\text{min}$
Nano LC	75 μm	300 nL/min

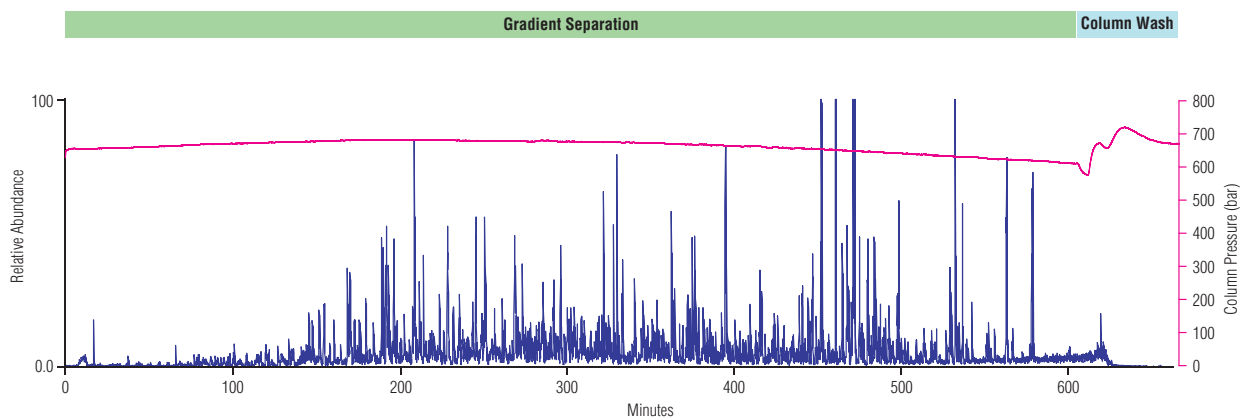


Figure 1: Ultrahigh resolution separation of an *E. coli* cell lysate tryptic digest sample on a 75 μm i.d. \times 50 cm Acclaim PepMap RSLC, 2 μm C18 column. A peak capacity of 750 was obtained using a flow rate of 270 nL/min and detection with ion-trap MS

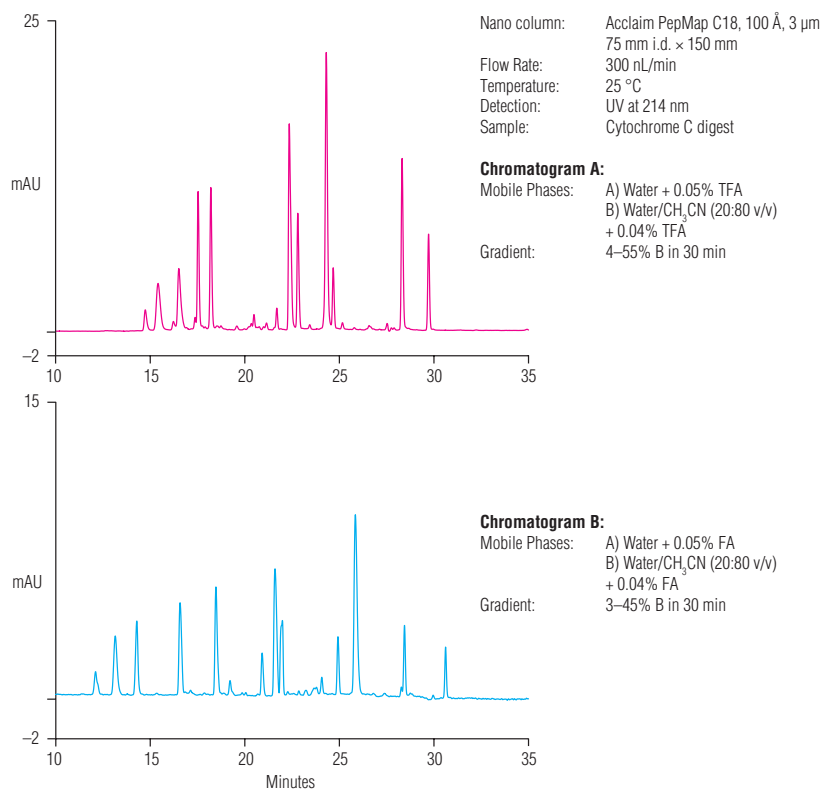


Figure 2: Comparison of the separation of cytochrome c with TFA (Chromatogram A) and FA (Chromatogram B) as ion-pairing agent in the mobile phase, respectively

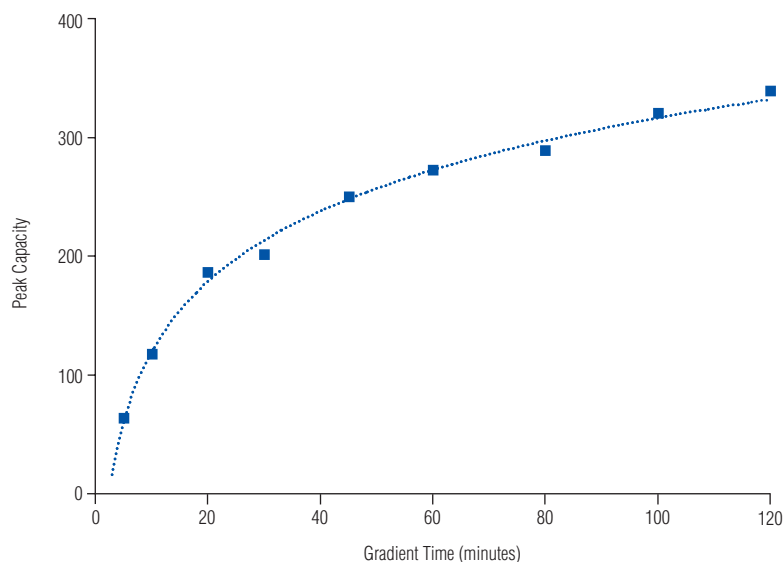


Figure 3: Effect of gradient time on peak capacity obtained on a 75 µm i.d. × 15 cm Acclaim PepMap nano LC column at a flow rate of 300 nL/min and a column temperature of 60 °C

Minimizing Ion-Suppression Effects with TFA-Free LC-MS

Acclaim PepMap is a silica-based C18 stationary phase with virtually zero silanophilic activity, resulting in the superior separation of peptides with minimal band broadening.

Consequently, the need for trifluoroacetic acid (TFA) in peptide mapping is no longer required, allowing the use of solvent additives, such as formic acid (FA), with substantially lower signal suppression and consequently higher sensitivity with mass spectrometric detection.

Acclaim PepMap Trap Columns for Desalting and Preconcentration

Trap columns are typically applied for the desalting of peptides before LC separation with MS detection, thus allowing fast sample preconcentration and clean-up of large volume injections.

The columns are tailored to work with the UltiMate 3000 Proteomics MDLC system and provide the highest efficiencies for one-dimensional peptide mapping experiments and 2D-LC analyses. Trap columns are available in two formats:

- Stainless steel cartridges to provide maximum robustness.
- Fused silica to provide the highest chromatographic performance.

Varying Column Lengths for Fast, High Efficiency nano LC Peptide Separations

The Acclaim PepMap nano LC column lengths target fast or high resolution peptide separation. The peak capacity and consequently the resolution between the peptide fragments depends strongly on the LC conditions applied, such as gradient time. The effect of gradient time on peak capacity for a 15 cm column is shown in Figure 3. Within 2 h, a peak capacity of 340 can be reached.

The 75 μm i.d. \times 5 cm Acclaim PepMap nano LC column targets fast peptide separations for relatively simple peptide mixtures. The 15 cm column is the best compromise between resolution, analysis time, and sensitivity for LC-MS peptide mapping. For more demanding peptide separations, the 75 μm i.d. \times 25 cm nano LC column is a good option. The 75 μm i.d. \times 50 cm Acclaim PepMap nano LC columns are tailored for high-resolution peptide separations. The performance of the 50 cm long Acclaim PepMap nano LC column for separation of a 6-protein digest with excellent resolution between the different peptides is demonstrated in Figure 4.

Column Technology for Two-Dimensional LC Separations

For very complex proteomics samples, two-dimensional LC is recommended. The UltiMate 3000 RSLCnano system is designed for desalting and gradient separations of peptides/proteins and for off-line and on-line 2D-LC separations.

The set-up for off-line 2D-LC is easy to install, and kits containing all the connection tubing and columns are available and tuned for peptide separations. An off-line separation performed on a 1 mm i.d. first-dimension SCX column and a second-dimension 75 μm i.d. Acclaim PepMap nano LC column is shown in Figure 5.

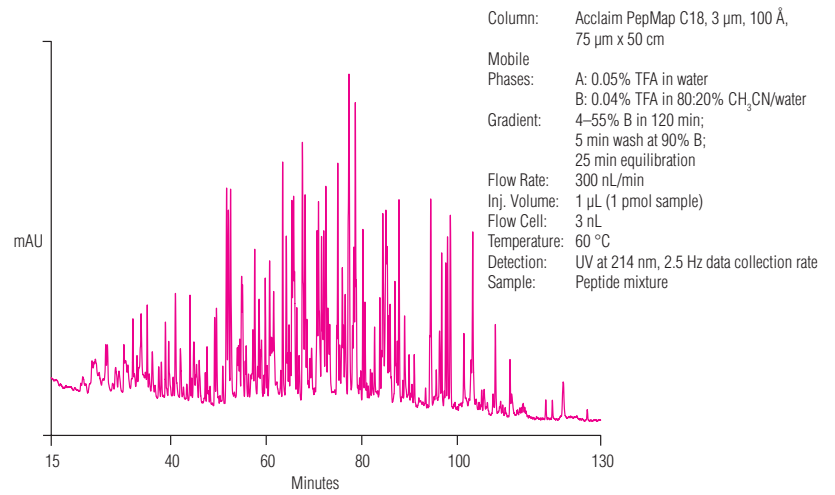


Figure 4: Example of a high peak-capacity separation of a complex peptide mixture obtained on a 50 cm long Acclaim PepMap nano LC column

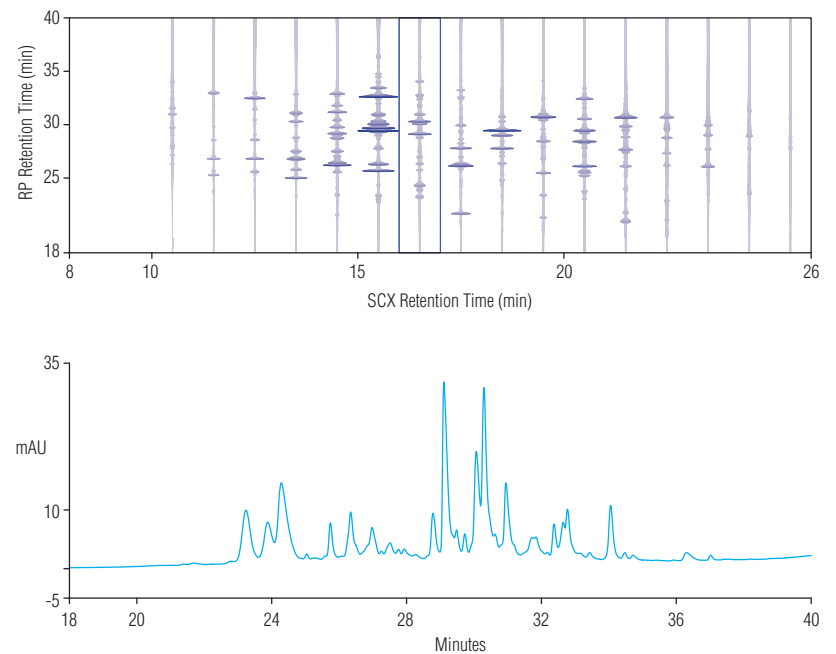


Figure 5: Off-line SCX \times RP separation example of a complex peptide mixture, using a 15 cm Acclaim PepMap column in the second dimension. The top diagram plots SCX fractions against the RP responses. The bottom chromatogram shows a representative SCX fraction separated using the RP column

nanoViper Fittings for Easy Column Installation and Exchange

All Acclaim PepMap RSLC columns and several Acclaim PepMap columns (see ordering information) are preassembled with nanoViper column inlet fittings for easy column installation. The nanoViper system is a fingertight fitting system that provides virtually zero-dead-volume connections. The nanoViper fitting is capable of withstanding pressures up to a 1000 bar and is compatible with third party valves and unions.

Key advantages of nanoViper fittings:

- Ease-of-use, no sleeve for small capillaries
- Tool-free assembly
- No column damage due to overtightening
- No experimental failure due to bad connections

Figure 6 shows a photo of the nanoViper column inlet fitting.



Figure 6: Photo of a nanoViper fitting

Acclaim PepMap and PepMap RSLC C18 Specifications		
Typical Applications	Peptide Analyses	
Base Material	Spherical silica, 100 Å pores	
Chemistry	C18, endcapped	
Column Dimensions	75 µm i.d. × 15 cm, 3 µm	75 µm i.d. × 15 cm, 2 µm
Recommended Flow Rate	200–500 nL/min	
Recommended Temperature	25–60 °C	
Maximum Pressure	500 bar	800 bar
Recommended Sample Quantity ¹	2 µg	
Maximum Sample Capacity ²	10 pmol	
pH Stability	2–8	
Solvent Compatibility	All common RP solvents	

¹The recommended sample quantity of the Acclaim PepMap100 75 µm i.d. columns is given in weight amount of a protein digest of bovine serum albumin.

²The maximum sample capacity is measured for tryptic peptides from cytochrome c digest tolerating a maximum 10% increase in peak width.

Ordering Information

Acclaim PepMap Columns	Part Number
Acclaim PepMap100 C18, 3 µm, 100 Å, 75 µm i.d. × 5 cm, nanoViper	164567
Acclaim PepMap100 C18, 3 µm, 100 Å, 75 µm i.d. × 15 cm	160321
Acclaim PepMap100 C18, 3 µm, 100 Å, 75 µm i.d. × 15 cm, nanoViper	164568
Acclaim PepMap100 C18, 3 µm, 100 Å, 75 µm i.d. × 25 cm	164261
Acclaim PepMap100 C18, 3 µm, 100 Å, 75 µm i.d. × 25 cm, nanoViper	164569
Acclaim PepMap100 C18, 3 µm, 100 Å, 75 µm i.d. × 50 cm, nanoViper	164570
Acclaim PepMap100 C18, 3 µm, 100 Å, 300 µm i.d. × 15 cm, nanoViper	164571
Acclaim PepMap100 C18, 3 µm, 100 Å, 1 mm i.d. × 15 cm, nanoViper	164572

Acclaim PepMap RSLC Columns	Part Number
Acclaim PepMap RSLC C18, 2 µm, 100 Å, 50 µm i.d. × 5 cm, nanoViper	164561
Acclaim PepMap RSLC C18, 2 µm, 100 Å, 50 µm i.d. × 15 cm, nanoViper	164562
Acclaim PepMap RSLC C18, 2 µm, 100 Å, 75 µm i.d. × 5 cm, nanoViper	164563
Acclaim PepMap RSLC C18, 2 µm, 100 Å, 75 µm i.d. × 15 cm, nanoViper	164534
Acclaim PepMap RSLC C18, 2 µm, 100 Å, 75 µm i.d. × 25 cm, nanoViper	164536
Acclaim PepMap RSLC C18, 2 µm, 100 Å, 75 µm i.d. × 50 cm, nanoViper	164540
Acclaim PepMap RSLC C18, 2 µm, 100 Å, 300 µm i.d. × 5 cm, nanoViper	164560
Acclaim PepMap RSLC C18, 2 µm, 100 Å, 300 µm i.d. × 15 cm, nanoViper	164537

Ordering Information (continued)

Trap Columns	Part Number
Acclaim PepMap100 C18, 5 µm, 100 Å, 300 µm i.d. × 1 mm (set of 5 for nano LC)	160428
Acclaim PepMap100 C18, 5 µm, 100 Å, 300 µm i.d. × 5 mm (set of 5 for nano LC)	160454
Acclaim PepMap100 C18, 5 µm, 100 Å, 1 mm i.d. × 5 mm (set of 5)	160434
Acclaim PepMap100 C18, 5 µm, 100 Å, 1 mm i.d. × 15 mm (set of 5)	160438

Trap Column Holders	Part Number
30 µm ID x 10 cm 1/32" connection tubing with nanoViper fittings, (set of 2)	164648
µ-Precolumn holder, 5 mm, with 30 µm i.d. connecting tubing, nanoViper fittings	164649
µ-Precolumn holder, 15 mm, with 75 µm i.d. connecting tubing, nanoViper fittings	164650

Nano Trap Columns	Part Number
Nano Trap Column, 100 µm i.d. × 1 cm, packed with Acclaim PepMap100 C18, 5 µm, 100 Å, (set of 2)	164197
Nano Trap Column, 100 µm i.d. × 2 cm, packed with Acclaim PepMap100 C18, 5 µm, 100 Å, (set of 2)	164199
Nano Trap Column, 100 µm i.d. × 2 cm, packed with Acclaim PepMap100 C18, 5 µm, 100 Å, (set of 2), nanoViper	164564
Nano Trap Column, 200 µm i.d. × 1 cm, packed with Acclaim PepMap100 C18, 5 µm, 100 Å, (set of 2)	164212
Nano Trap Column, 200 µm i.d. × 2 cm, packed with Acclaim PepMap100 C18, 5 µm, 100 Å, (set of 2)	164213
Nano Trap Column, 75 µm i.d. × 2 cm, packed with Acclaim PepMap100 C18, 3 µm, 100 Å, (set of 2), nanoViper	164535

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