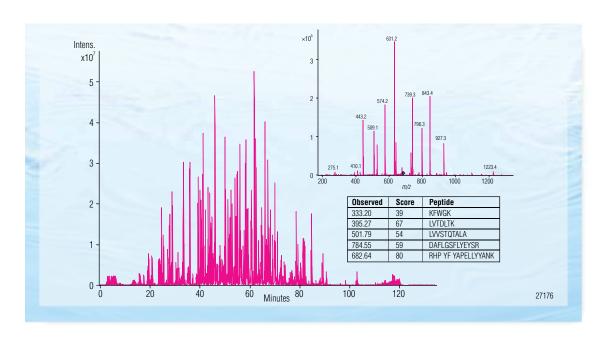


# Acclaim PepMap and Acclaim PepMap RSLC Columns for High-Resolution Peptide Mapping



Dionex 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
- nanoViper<sup>™</sup> fingertight fittings 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 ultrahigh-resolution peptide separations. The 2 µm particles of this stationary phase yield smaller peak widths.

A high pressure nano LC system, such as the 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 μL/min
Capillary LC	300 μm	4 μL/min
Nano LC	75 μm	300 nL/min

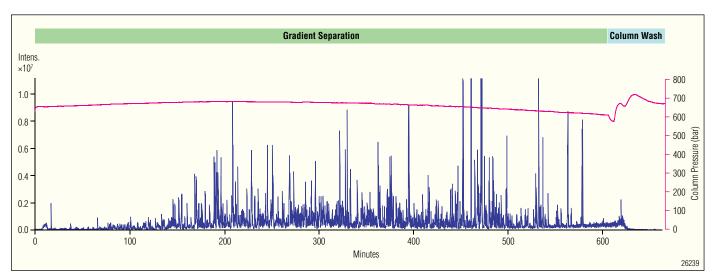


Figure 1. Ultrahigh resolution separation of an E. coli cell lysate tryptic digest sample on a 75  $\mu$ m i.d.  $\times$  50 cm Acclaim PepMap RSLC, 2  $\mu$ m C18 column. A peak capacity of 750 was obtained using a flow rate of 270 nL/min and detection with ion-trap MS.

#### 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 TFA in peptide mapping is no longer required, allowing the use of solvent additives, such as formic acid, 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 Dionex 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.

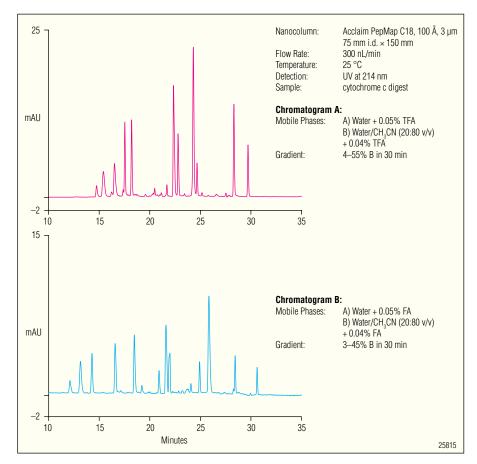


Figure 2. Comparison of the separation of cytochrome c with TFA (Chromatogram A) and FA (Chromatogram B) as ion-pairing agent in the mobile phase, respectively.

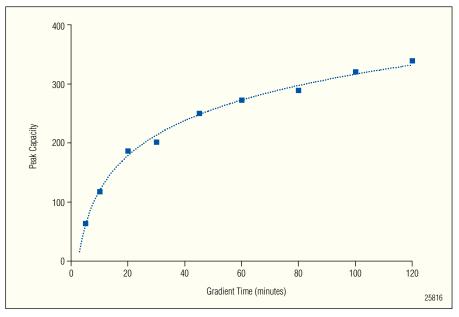


Figure 3. Effect of gradient time on peak capacity obtained on a 75  $\mu$ m i.d.  $\times$  15 cm Acclaim PepMap nano LC column at a flow rate of 300 nL/min and a column temperature of 60 °C.

The 75  $\mu$ m i.d.  $\times$  5 cm Acclaim PepMap nano LC column targets highspeed 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. × 25 cm nanoLC column is a good option. The 75  $\mu$ m i.d.  $\times$  50 cm Acclaim PepMap nanoLC columns are tailored for high-resolution peptide separations. The performance of the 50 cmlong 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 nanoLC column is shown in Figure 5.

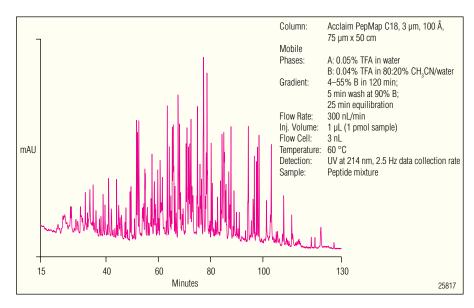


Figure 4. Example of a high peak-capacity separation of a complex peptide mixture obtained on a 50 cm long Acclaim PepMap nanoLC column.

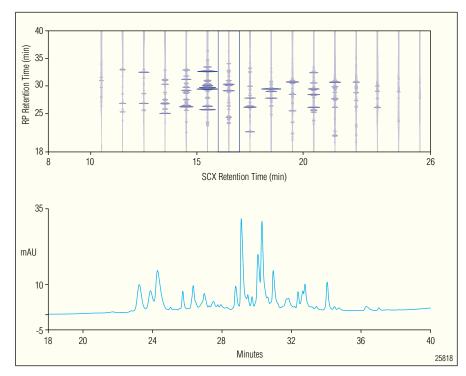


Figure 5. Off-line SCX × RP separation example of a complex peptide mixture, using a 15 cm Acclaim PepMap column in the second dimension. The top diagram plots SCX fractions against the RP responses. The bottom chromatogram shows a representative SCX fraction separated using the RP column.

### 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 zero-dead-volume connections. The nanoViper fitting is capable of withstanding pressures up to a 1000 bars 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 connections

Figure 6 shows a photo of the nanoViper column inlet 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 <sup>1</sup>	2 μg			
Maximum Sample Capacity <sup>2</sup>	10 pmol			
pH Stability	2–8			
Solvent Compatibility	All common RP solvents			

 $<sup>^{1}</sup>$ The recommended sample quantity of the Acclaim PepMap100 75  $\mu m$  i.d. columns is given in weight amount of a protein digest of bovine serum albumin.

<sup>&</sup>lt;sup>2</sup>The maximum sample capacity is measured for tryptic peptides from cytochrome c digest tolerating a maximum 10% increase in peak width.



Figure 6. Photo of a nanoViper fitting.

#### 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.

Acclaim PepMap Columns	Part Number
Acclaim PepMap100, 3 μm, 100 Å, 75 μm i.d. × 5 cm	160316
Acclaim PepMap100, 3 μm, 100 Å, 75 μm i.d. × 5 cm, nanoViper	164567
Acclaim PepMap100, 3 μm, 100 Å, 75 μm i.d. × 15 cm	160321
Acclaim PepMap100, 3 μm, 100 Å, 75 μm i.d. × 15 cm, nanoViper	164568
Acclaim PepMap100, 3 μm, 100 Å, 75 μm i.d. × 25 cm	164261
Acclaim PepMap100, 3 μm, 100 Å, 75 μm i.d. × 25 cm, nanoViper	164569
Acclaim PepMap100, 3 μm, 100 Å, 75 μm i.d. × 50 cm	164451
Acclaim PepMap100, 3 μm, 100 Å, 75 μm i.d. × 50 cm, nanoViper	164570
Acclaim PepMap100, 3 μm, 100 Å, 300 μm i.d. × 5 cm	
Acclaim PepMap100, 3 μm, 100 Å, 300 μm i.d. × 15 cm	160295
Acclaim PepMap100, 3 μm, 100 Å, 300 μm i.d. × 15 cm, nanoViper	
Acclaim PepMap100, 3 μm, 100 Å, 1 mm i.d. × 5 cm	160277
Acclaim PepMap100, 3 μm, 100 Å, 1 mm i.d. × 15 cm	160282
Acclaim PepMap100, 3 μm, 100 Å, 1 mm i.d. × 15 cm, nanoViper	164572

ORDERING INFORMATION (CONT'D)		
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	
Trap Columns	Part Number	
Acclaim PepMap100, 5 μm, 100 Å, 300 μm i.d. × 1 mm (set of 5 for nanoLC)	160458	
Acclaim PepMap100, 5 μm, 100 Å, 300 μm i.d. × 5 mm (set of 5 for nanoLC)	160454	
Acclaim PepMap100, 5 μm, 100 Å, 1 mm i.d. × 5 mm (set of 5)	160434	
Acclaim PepMap100, 5 μm, 100 Å, 1 mm i.d. × 15 mm (set of 5)	160438	
Trap Column Holders	Part Number	
Trap Column Holder, 1 mm with connection tubing (30 μm i.d.)	160461	
Trap Column Holder, 5 mm with connection tubing (30 μm i.d.)	160431	
Trap Column Holder, 15 mm with connection tubing (60 μm i.d.)	160432	
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, $100~\mu m$ i.d. $\times~2~cm$ , packed with Acclaim PepMap $100~C18, 5~\mu m$ , $100~Å$ , (set of 2), nanoViper $164564~cm$		
Nano Trap Column, 200 $\mu m$ i.d. $\times$ 1 cm, packed with Acclaim PepMap100 C18, 5 $\mu m$ , 100 Å, (set of 2)		
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), nano	Viper 164535	

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