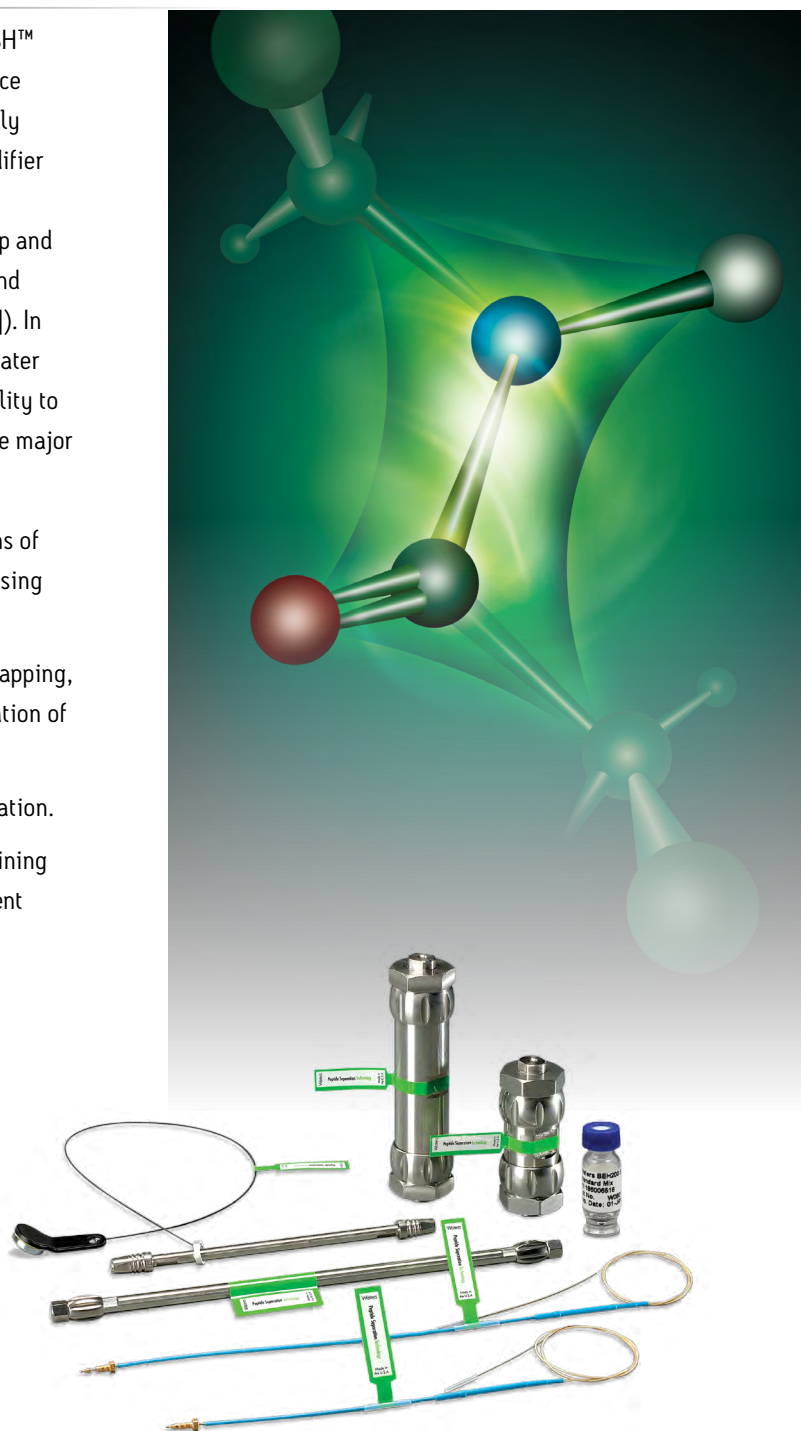


## CSH130 C<sub>18</sub> Columns for Peptide Separations

Charged Surface Hybrid particles deliver superior peptide separations in LC or LC-MS applications

Waters patented synthesis process for its Charged Surface Hybrid (CSH™ Technology) particles imparts a low level positive charge to the surface of each particle. CSH Technology allows the columns to be successfully used with standard TFA-containing eluents or with a weaker acid modifier such as formic acid. This means users no longer have to compromise between selecting a reversed-phase eluent that delivers needed sharp and symmetrical separated peaks (e.g., 0.1% trifluoroacetic acid [TFA]) and one that minimizes reduction of MS signal (e.g., 0.1% formic acid [FA]). In similar fashion, the ability of the CSH130 C<sub>18</sub> chemistry to accept greater peptide mass loads than on many other columns will enhance the ability to detect potentially important low level constituents contained with the major component(s) of interest.

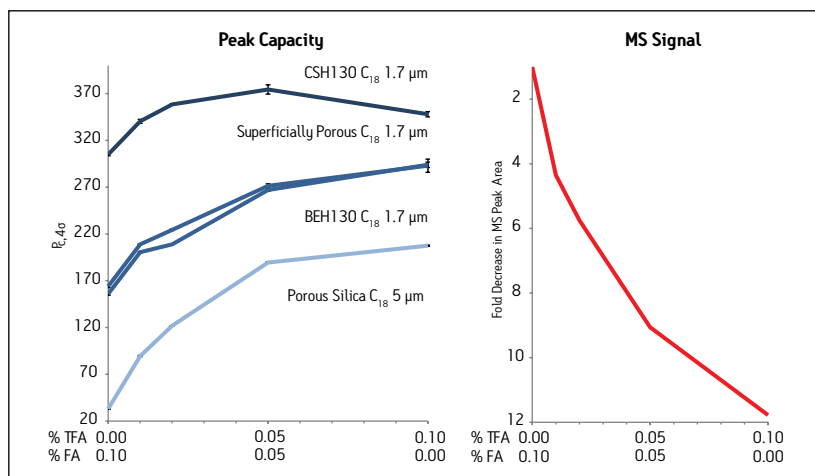
- Outstanding peak capacities in HPLC, UPLC®, or LC-MS separations of peptide and small proteins up to approximately 10,000 Daltons using either 0.1% FA- or 0.1% TFA-containing eluents.
- Excellent mass loading of complex peptide samples for peptide mapping, proteomics applications, or for the analysis and lab-scale purification of synthetic peptides.
- Scalable chemistries from nano flow analysis to lab-scale purification.
- QC tested with a cytochrome *c* tryptic digest using 0.1% FA-containing eluents to help ensure column-to-column reproducibility for consistent performance in validated methods.



## SUPERIOR PERFORMANCE IN EITHER FA- OR TFA-CONTAINING ELUENTS

Waters CSH130 C<sub>18</sub> particles contain a low and carefully-defined concentration of positive charges that yield comparatively excellent peak shape for peptides using either FA- or TFA-containing mobile phases. The fact that the performance of a CSH130 C<sub>18</sub> column exhibits little dependence on strong ion pairing agents makes it ideal for LC or LC-MS applications.

### Comparative Averaged Peptide Peak Capacities on Selected Reversed-Phase Columns with Differing Concentrations of FA and TFA

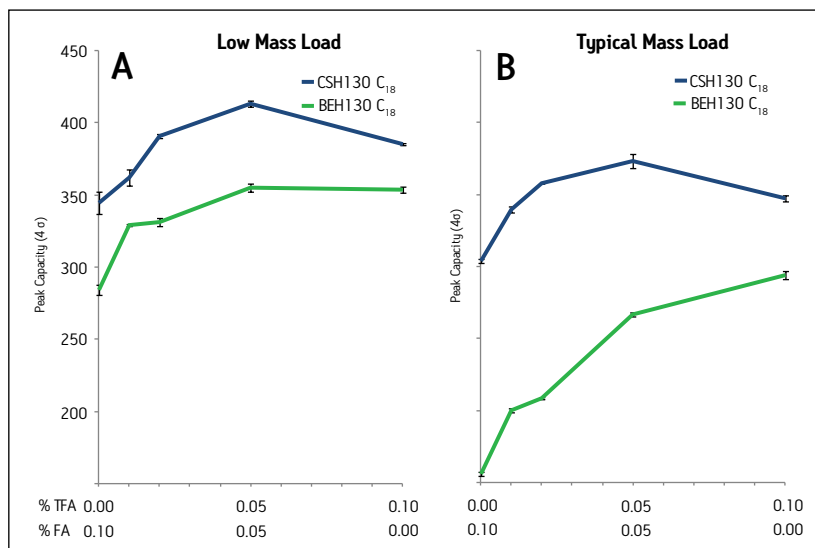


Effect of TFA on peak capacity and MS signal. (A) Peak capacity as a function of acid modifier. Values were derived from two replicates. (B) Fold decrease in MS peak area as a function of acid modifier. Waters MassPREP™ Peptide Standard Mixture (P/N 186002337) was used in study.

## EXCELLENT MASS LOADING OF COMPLEX PEPTIDE SAMPLES

One of the inherent performance advantages of CSH technology is improved sample mass loadability—how much analyte can be loaded onto a column before peak shape deteriorates. Clearly, at typical mass loads, CSH130 C<sub>18</sub> delivers remarkably better performance than many existing C<sub>18</sub> offerings. When loading 10x less sample, the difference in performance was less pronounced. Improved peptide mass loadability is an excellent column asset for challenging separations, particularly those that involve mixtures comprised of species present at vastly different concentrations.

### Comparative Averaged Peptide Peak Capacities on CSH130 vs. BEH130-based, C<sub>18</sub> Columns (2.1 x 150 mm) at Two Peptide Mass Loads and Differing Concentrations of FA and TFA

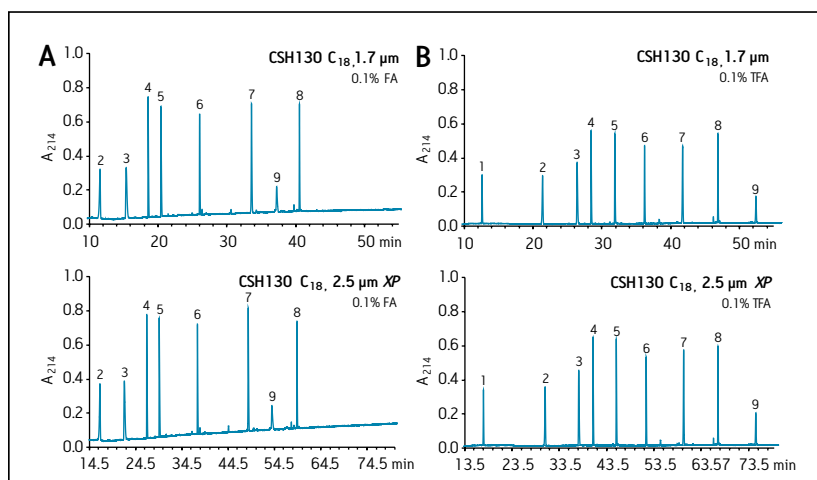


Effect of Peptide Mass Load in FA, TFA, and FA/TFA blends on peak capacity. (A) approx. 0.06 μg peptide mixture sample load. (B) approx. 0.6 μg peptide mixture. Values were derived from two replicates. Waters MassPREP Peptide Standard Mixture (P/N 186002337) was used in study.

## SCALABLE COLUMN OFFERINGS FOR HPLC- OR UPLC-BASED SEPARATIONS

There remains a need for columns that are compatible with HPLC instrumentation. Low dispersion LC instrumentation is recommended in order to get full performance from a well-packed column containing 1.7  $\mu\text{m}$  particles. The recent introduction of Waters eXtended Performance [XP] columns packed with 2.5  $\mu\text{m}$  particles has allowed scientists to improve their existing HPLC instrumentation productivity. The high peak capacity peptide separations obtained with a CSH130 C<sub>18</sub>, 1.7  $\mu\text{m}$  column could, for instance, be successfully scaled to a CSH130 C<sub>18</sub>, 2.5  $\mu\text{m}$  XP column by correct scaling of the flow rate and the gradient time. As shown below, CSH technology can therefore be readily employed for high peak capacity peptide separations using either UPLC or HPLC instrumentation.

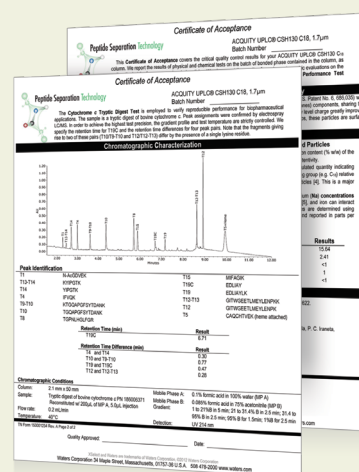
### Comparative Separation of MassPREP Peptide Standard Mixture on ACQUITY UPLC CSH130 C<sub>18</sub>, 1.7 $\mu\text{m}$ vs. XSelect CSH130 C<sub>18</sub>, 2.5 $\mu\text{m}$ offering in 0.1% FA- or 0.1% TFA-Containing Eluents



Chromatograms of Waters MassPREP Peptide Standard Mixture (P/N 186002337) obtained with (A) 0.1% FA and (B) 0.1% TFA mobile phases. The method for the CSH130 C<sub>18</sub>, 2.5  $\mu\text{m}$  XP column was scaled from the method for the CSH130 C<sub>18</sub>, 1.7  $\mu\text{m}$  column by decreasing flow rate and increasing gradient time by a factor of 1.5. Backpressure on the XSelect<sup>®</sup> CSH130 C<sub>18</sub>, 2.5  $\mu\text{m}$  XP, 2.1 x 150 mm column was 3,000 psi while a backpressure value of 8,000 psi was generated on the ACQUITY UPLC<sup>®</sup> CSH130 C<sub>18</sub>, 1.7  $\mu\text{m}$ , 2.1 x 150 mm offering.

## INCREASED ASSURANCE WITH WATERS PEPTIDE SEPARATION TECHNOLOGY (PST) COLUMNS

Each batch of Waters CSH130 C<sub>18</sub> material is QC tested with a cytochrome c tryptic digest to help ensure column-to-column reproducibility. This important quality test is performed with a gradient separation and 0.1% FA-containing eluents that challenges batch performance to help ensure consistent column performance for research or validated method requirements.



Certificate of Analysis information includes a labeled chromatogram of the gradient separation of a tryptic digest of bovine cytochrome c (Waters P/N 186006371) using 0.1% FA containing eluents. The same protein digest test mixture can be purchased by customers to ensure CSH130 C<sub>18</sub> column performance.

## ORDERING INFORMATION

ACQUITY UPLC and XSelect CSH130 C<sub>18</sub> Analytical Columns

Kit Part Number <sup>(a)</sup>	Part Number	Description
<a href="#">176003061</a>	<a href="#">186006933</a>	ACQUITY UPLC CSH130 C <sub>18</sub> , 1.0 x 50 mm, 1.7 µm Column
<a href="#">176003062</a>	<a href="#">186006934</a>	ACQUITY UPLC CSH130 C <sub>18</sub> , 1.0 x 100 mm, 1.7 µm Column
<a href="#">176003063</a>	<a href="#">186006935</a>	ACQUITY UPLC CSH130 C <sub>18</sub> , 1.0 x 150 mm, 1.7 µm Column
<a href="#">176003064</a>	<a href="#">186006936</a>	ACQUITY UPLC CSH130 C <sub>18</sub> , 2.1 x 50 mm, 1.7 µm Column
<a href="#">176003065</a>	<a href="#">186006937</a>	ACQUITY UPLC CSH130 C <sub>18</sub> , 2.1 x 100 mm, 1.7 µm Column
<a href="#">176003066</a>	<a href="#">186006938</a>	ACQUITY UPLC CSH130 C <sub>18</sub> , 2.1 x 150 mm, 1.7 µm Column
176003067	<a href="#">186006939</a>	ACQUITY UPLC CSH130 C <sub>18</sub> 2.1 x 50 mm, 1.7 µm VanGuard™ Column, 3/pk
<a href="#">176003068</a>	<a href="#">186006940</a>	ACQUITY UPLC CSH130 C <sub>18</sub> , 2.1 x 150 mm, 1.7 µm Column Method Validation Kit <sup>(b)</sup>
<a href="#">176003069</a>	<a href="#">186006941</a>	XSelect CSH130 C <sub>18</sub> , 2.1 x 50 mm XP, 2.5 µm Column
<a href="#">176003070</a>	<a href="#">186006942</a>	XSelect CSH130 C <sub>18</sub> , 2.1 x 100 mm XP, 2.5 µm Column
<a href="#">176003071</a>	<a href="#">186006943</a>	XSelect CSH130 C <sub>18</sub> , 2.1 x 150 mm XP, 2.5 µm Column
176003072	<a href="#">186006944</a>	XSelect CSH130 C <sub>18</sub> , 2.1 x 5 mm 2.5 µm VanGuard Column, 3/pk
<a href="#">176003073</a>	<a href="#">186006945</a>	XSelect CSH130 C <sub>18</sub> , 2.1 x 100 mm XP, 2.5 µm Column Method Validation Kit <sup>(b)</sup>
<a href="#">176003074</a>	<a href="#">186006946</a>	XSelect CSH130 C <sub>18</sub> , 4.6 x 50 mm XP, 2.5 µm Column
<a href="#">176003075</a>	<a href="#">186006947</a>	XSelect CSH130 C <sub>18</sub> , 4.6 x 100 mm XP, 2.5 µm Column
<a href="#">176003093</a>	<a href="#">186007038</a>	XSelect CSH130 C <sub>18</sub> , 4.6 x 150 mm XP, 2.5 µm Column
<a href="#">176003076</a>	<a href="#">186006966</a>	XSelect CSH130 C <sub>18</sub> , 4.6 x 100 mm XP, 2.5 µm Column Method Validation Kit <sup>(b)</sup>
<a href="#">176003077</a>	<a href="#">186006950</a>	XSelect CSH130 C <sub>18</sub> , 2.1 x 50 mm, 3.5 µm Column
<a href="#">176003078</a>	<a href="#">186006951</a>	XSelect CSH130 C <sub>18</sub> , 2.1 x 100 mm, 3.5 µm Column
<a href="#">176003079</a>	<a href="#">186006952</a>	XSelect CSH130 C <sub>18</sub> , 2.1 x 150 mm, 3.5 µm Column
<a href="#">176003080</a>	<a href="#">186006953</a>	XSelect CSH130 C <sub>18</sub> , 2.1 x 100 mm, 3.5 µm Column Method Validation Kit <sup>(b)</sup>
176003081	<a href="#">186006954</a>	XSelect CSH130 C <sub>18</sub> , 2.1 x 10 mm, 3.5 µm Guard Column, 2/pk <sup>(c)</sup>
<a href="#">176003082</a>	<a href="#">186006955</a>	XSelect CSH130 C <sub>18</sub> , 4.6 x 50 mm, 3.5 µm Column
<a href="#">176003083</a>	<a href="#">186006956</a>	XSelect CSH130 C <sub>18</sub> , 4.6 x 100 mm, 3.5 µm Column
<a href="#">176003084</a>	<a href="#">186006957</a>	XSelect CSH130 C <sub>18</sub> , 4.6 x 150 mm, 3.5 µm Column
176003085	<a href="#">186006958</a>	XSelect CSH130 C <sub>18</sub> , 4.6 x 20 mm, 3.5 µm Guard Column, 2/pk <sup>(d)</sup>
<a href="#">176003086</a>	<a href="#">186006959</a>	XSelect CSH130 C <sub>18</sub> , 4.6 x 100 mm, 3.5 µm Column Method Validation Kit <sup>(b)</sup>
	<a href="#">186006371</a>	Cytochrome c Digestion Standard

<sup>(a)</sup> Kit includes CSH130 C<sub>18</sub> Column plus one vial of Cytochrome c Digestion Standard (P/N [186006371](#))

<sup>(b)</sup> Each kit contains 3 columns from 3 different batches of CSH130 C<sub>18</sub> material

<sup>(c)</sup> Requires 2.1 x 10 mm Universal Sentry Guard Holder, P/N [WAT097958](#)

<sup>(d)</sup> Requires 4.6 x 20 mm Universal Sentry Guard Holder, P/N [WAT046910](#)

## ORDERING INFORMATION

ACQUITY UPLC M-Class CSH C<sub>18</sub>, 130Å Columns for Nano- and Capillary-Scale UPLC Separations

Part Number	Description
<a href="#">186007479</a>	ACQUITY UPLC M-Class Peptide CSH C <sub>18</sub> , 130Å, 1.7 µm, 100 µm x 100 mm Column, 1/pkg
<a href="#">186007480</a>	ACQUITY UPLC M-Class Peptide CSH C <sub>18</sub> , 130Å, 1.7 µm, 150 µm x 100 mm Column, 1/pkg
<a href="#">186007513</a>	ACQUITY UPLC M-Class Peptide CSH C <sub>18</sub> , 130Å, 1.7 µm, 150 µm x 50 mm Column, 1/pkg
<a href="#">186007514</a>	ACQUITY UPLC M-Class Peptide CSH C <sub>18</sub> , 130Å, 1.7 µm, 150 µm x 150 mm Column, 1/pkg
<a href="#">186007475</a>	ACQUITY UPLC M-Class Peptide CSH C <sub>18</sub> , 130Å, 1.7 µm, 75 µm x 100 mm Column, 1/pkg
<a href="#">186007476</a>	ACQUITY UPLC M-Class Peptide CSH C <sub>18</sub> , 130Å, 1.7 µm, 75 µm x 150 mm Column, 1/pkg
<a href="#">186007477</a>	ACQUITY UPLC M-Class Peptide CSH C <sub>18</sub> , 130Å, 1.7 µm, 75 µm x 200 mm Column, 1/pkg
<a href="#">186007478</a>	ACQUITY UPLC M-Class Peptide CSH C <sub>18</sub> , 130Å, 1.7 µm, 75 µm x 250 mm Column, 1/pkg
<a href="#">186007561</a>	ACQUITY UPLC M-Class Peptide CSH C <sub>18</sub> , 130Å, 1.7 µm, 300 µm x 50 mm Column, 1/pkg
<a href="#">186007562</a>	ACQUITY UPLC M-Class Peptide CSH C <sub>18</sub> , 130Å, 1.7 µm, 300 µm x 100 mm Column, 1/pkg
<a href="#">186007563</a>	ACQUITY UPLC M-Class Peptide CSH C <sub>18</sub> , 130Å, 1.7 µm, 300 µm x 150 mm Column, 1/pkg

XSelect CSH130 C<sub>18</sub> Preparative Columns

Part Number	Description
<a href="#">186007076</a>	XSelect CSH130 C <sub>18</sub> , 4.6 x 50mm, 5 µm Column <sup>(e)</sup>
<a href="#">186007077</a>	XSelect CSH130 C <sub>18</sub> , 4.6 x 100mm, 5 µm Column <sup>(e)</sup>
<a href="#">186007078</a>	XSelect CSH130 C <sub>18</sub> , 4.6 x 150mm, 5 µm Column <sup>(e)</sup>
<a href="#">186007018</a>	XSelect CSH130 C <sub>18</sub> , 10 x 50 mm, 5 µm Column
<a href="#">186007032</a>	XSelect CSH130 C <sub>18</sub> , 10 x 100 mm, 5 µm Column
<a href="#">186007016</a>	XSelect CSH130 C <sub>18</sub> , 10 x 150 mm, 5 µm Column
<a href="#">186007017</a>	XSelect CSH130 C <sub>18</sub> , 10 x 250 mm, 5 µm Column
<a href="#">186007015</a>	XSelect CSH130 C <sub>18</sub> , 10 x 10 mm, 5 µm Guard <sup>(f)</sup>
<a href="#">700001436</a>	Replacement O-Ring, 10 mm (2/pk)
<a href="#">186007022</a>	XSelect CSH130 C <sub>18</sub> , 19 x 50 mm, 5 µm OBD Column
<a href="#">186007020</a>	XSelect CSH130 C <sub>18</sub> , 19 x 100 mm, 5 µm OBD Column
<a href="#">186007021</a>	XSelect CSH130 C <sub>18</sub> , 19 x 150 mm, 5 µm OBD Column
<a href="#">186007031</a>	XSelect CSH130 C <sub>18</sub> , 19 x 250 mm, 5 µm OBD Column
<a href="#">186007019</a>	XSelect CSH130 C <sub>18</sub> , 19 x 10 mm, 5 µm Guard <sup>(g)</sup>
<a href="#">700001020</a>	Replacement O-Ring, 19 mm (2/pk)
<a href="#">186007026</a>	XSelect CSH130 C <sub>18</sub> , 30 x 50 mm, 5 µm OBD Column
<a href="#">186007025</a>	XSelect CSH130 C <sub>18</sub> , 30 x 100 mm, 5 µm OBD Column
<a href="#">186007023</a>	XSelect CSH130 C <sub>18</sub> , 30 x 150 mm, 5 µm OBD Column
<a href="#">186007024</a>	XSelect CSH130 C <sub>18</sub> , 30 x 250 mm, 5 µm OBD Column
<a href="#">186007030</a>	XSelect CSH130 C <sub>18</sub> , 50 x 50 mm, 5 µm OBD Column
<a href="#">186007027</a>	XSelect CSH130 C <sub>18</sub> , 50 x 100 mm, 5 µm OBD Column
<a href="#">186007028</a>	XSelect CSH130 C <sub>18</sub> , 50 x 150 mm, 5 µm OBD Column
<a href="#">186007029</a>	XSelect CSH130 C <sub>18</sub> , 50 x 250 mm, 5 µm OBD Column

<sup>(e)</sup> For use in developing lab-scale preparative chromatography

<sup>(f)</sup> Requires 10 x 10 mm Cartridge Holder, Part No. [289000779](#)

<sup>(g)</sup> Requires 19 x 10 mm Cartridge Holder, Part No. [186000709](#)

## References:

- 1) "Increasing Peak Capacity in Reversed Phase Peptide Separations with Charged Surface Hybrid (CSH) C<sub>18</sub> Columns"  
M.A. Lauber, S.M. Koza, K.J. Fountain  
Waters Application Note, [720004568EN](#), 2013
- 2) "Peptide Mapping and Small Protein Separations with Charged Surface Hybrid (CSH) C<sub>18</sub> and TFA-Free Mobile Phases"  
M.A. Lauber, S.M. Koza, K.J. Fountain  
Waters Application Note, [720004571EN](#), 2013
- 3) "Charged Surface Hybrid (CSH) Technology and Its Use in Liquid Chromatography"  
P.C. Iraneta, K.D. Wyndham, D.R. McCabe, and T.H. Walter  
Waters White Paper, [720003929EN](#), 2011
- 4) "Characterization and evaluation of C18 HPLC stationary phases based on ethyl-bridged hybridorganic/inorganic particles"  
Wyndham, K. D.; O'Gara, J. E.; Walter, T. H.; Glose, K. H.; Lawrence, N. L.; Alden, B. A.; Izzo, G. S.; Hudalla, C. J.; Iraneta, P. C., Anal Chem 2003, 75 (24), 6781-8.

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