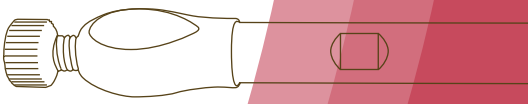
AERIS™

Column Care Guide



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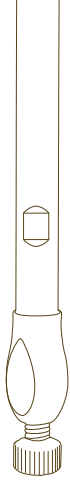

INTRODUCTION

Aeris WIDEPORE and Aeris PEPTIDE columns are specifically designed for the reversed phase separation of biomolecules. By optimizing particle size, shell thickness, and bonding chemistry, Aeris columns provide excellent resolution of proteins and peptides in a wide range of molecular weights.

The information and recommendations contained in this manual are designed to guide you in the care and use of your column, but should not be considered absolute. Please follow the instructions listed to maximize column performance and lifetime.

For additional information contact your local Phenomenex technical representative or check online at:

WWW.PHENOMENEX.COM/AERIS

AERIS WIDEPORE

Aeris WIDEPORE is a 3.6 µm, large pore core-shell column specifically designed for the reversed phase separation of large biomolecules where standard pore (~100 Å) sizes are inadequate. Aeris WIDEPORE columns provide excellent resolution of proteins larger than 10 Kilodaltons and oligonucleotides greater than 60 mer in length. Three chemistries, XB-C18, XB-C8 and C4, provide different selectivities giving several core-shell options for developing applications.

AERIS PEPTIDE

Aeris PEPTIDE is a small pore core-shell column specifically designed for the reversed phase separation of smaller biomolecules, and comes in both 3.6 µm and 1.7 µm particle sizes. Aeris PEPTIDE columns provide excellent resolution for peptides smaller than 10 Kilodaltons. The XB-C18 chemistry provides unique selectivity, long lifetimes, and column stability.

COLUMN SPECIFICATION AND SHIPPING INFORMATION

Each Aeris column has been individually packed and tested to meet a demanding performance specification. A chromatogram of the column packing performance test is included with every Aeris column. The certificate of analysis lists the physical description of the Aeris column, a specific serial number for the column, and sorbent batch lot used in the manufacture of the column. The testing conditions used are also listed on the certificate of analysis as well as the performance results for each analyte in the test mixture. The test mixture used for verifying column performance (AL0-3045 for 4.6 mm ID Aeris WIDEPORE and Aeris PEPTIDE columns, AL0-8931 for 2.1 mm Aeris WIDEPORE columns) contains uracil, acetophenone, toluene and naphthalene (acenaphthalene is an additional component in AL0-8931). Separation parameters for the test mixture are used as a measure of column packing quality as well as a diagnostic of system performance. If the performance of your Aeris column is not similar to the certificate of analysis, please review the system optimization tips in this guide, contact your local Phenomenex representative, or check online at:

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Media Validation Document

Every Aeris column includes a media validation document which describes a partial list of particle specifications and phase selectivity tests performed on every batch of core-shell media used for Aeris columns.

UPON RECEIPT OF THE COLUMN

- Verify that the column received is the correct phase and dimension that was ordered
- Inspect the column and packing for any physical damage that might have occurred during shipping
- Test the column as soon as possible to verify performance using a recommended test standard
- Keep a record of the test chromatograms for performance monitoring of column and system operation.

OPERATING PARAMETERS

Column Installation

The arrow on the column tag indicates the flow direction. Phenomenex recommends the use of HPLC/ UHPLC Sure-Lok™ High Pressure PEEK™ male nut fittings for installation of Aeris columns on HPLC/ UHPLC systems. Either the one-piece (AQ0-8502, rated to 800 bar) or 3-piece (AQ0-8504, rated to 1300 bar) fittings are recommended for use with Aeris columns to provide a zero dead volume, high pressure leak-free connection.

MOBILE PHASE LIMITATIONS

Aeris columns are stable from pH 1.5 to 9 and can be used with typical reversed phase mobile phase solvents (water, acetonitrile, methanol, isopropanol, and tetrahydrofuran mixtures) and buffers (TFA, formic acid, acetic acid, etc).

Use only high purity reagents and high quality chromatography grade solvents to prepare mobile phase. Trace impurities can dramatically degrade column lifetime. Degas and filter all mobile phase prior to use. Ensure sample (and matrix) are soluble/ miscible with mobile phase. Immiscible solvents or buffer salt precipitation can permanently damage the column.

Avoid:

- Operating below pH 1.5 as this will hydrolyze the bonded phase
- Operating above pH 9 will dissolve the core-shell silica backbone.
- Immiscible solvents and buffers
- Sudden pressure changes

Operating Backpressure

3.6 µm columns: Because a larger core-shell particle is used for Aeris WIDEPORE and Aeris PEPTIDE columns it is unlikely that one will reach excessive backpressures anywhere near the optimal flow rate for Aeris 3.6 µm columns. However, for limitation purposes, Aeris 3.6 µm columns are rated for operation up to a maximum backpressure of 600 bar (8,700 psi). Long-term operations above 600 bar backpressure will result in shorter column lifetimes. Aeris 1.7 µm columns can be operated up to 1,000 bar (15,000 psi).

Operating Temperature

It has been recognized by several chromatographers that higher column temperatures can give improved resolution and thus, Aeris core-shell columns were designed to tolerate high column temperatures.

- Aeris WIDEPORE XB-C18 and XB-C8 columns were designed to operate at column temperatures up to 90 °C.
- Aeris WIDEPORE C4 columns can be operated up to 60 °C.
- Aeris PEPTIDE XB-C18 columns can be used up to 90 °C.

Note that most columns will have shorter column lifetime when operating close to their maximum temperature limit. Column lifetimes will be further reduced operating at high temperature close to the pH limits ≤ pH 2 or ≥ pH 8. When operating close to the pH limit of the Aeris columns, lower column temperatures are recommended for longer column lifetime.

Column Cleaning and Regeneration

If an increase in operating backpressure is observed, reverse flush the column (do not try this on other manufacturers' columns) with reduced flow rates indicated below:

Column ID	Flow Rate
2.1 mm	0.1 mL/min
4.6 mm	0.5 mL/min

Aeris columns are best flushed by following the below cleaning procedure:

- Replace solvent reservoirs with 100 % water and 100 % acetonitrile
- Perform a slow gradient (1-2 %/ min) from 5 % to 95 % acetonitrile and back to 5 % acetonitrile
- Replace solvent reservoir B with 100 % isopropanol (IPA)
- Perform a slow gradient (1-2 %/min) from 5 % to 95 % IPA and back to 5 % IPA (reduce flow rate if backpressure exceeds 350 bar).
- Replace mobile phase on solvent reservoirs, re-orientate the column in proper operating direction and re-equilibrate.

Column Storage

Column storage for a period longer than several days is recommended in 50 % acetonitrile in water. Mobile phase buffer should be flushed from the column with 10-20 column volumes of acetonitrile/ water prior to storage. After flushing the column, insert column end plugs securely to prevent evaporation and drying out of the column bed.

OPTIMIZING THE PERFORMANCE OF YOUR AERIS COLUMN

Flow Rate and Column Length

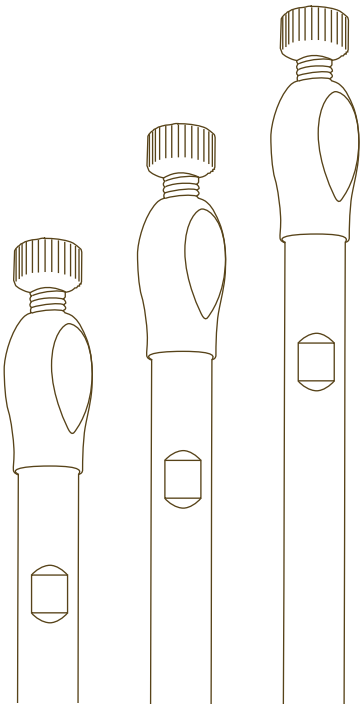
While small molecule separations on core-shell media usually require faster linear velocities for improved efficiency, for protein separations on Aeris columns maximum resolution is typically obtained at flow rate only slightly higher than existing fully porous media. Thus, one should start their method development using column dimensions and flow rates similar to existing protein separation methods. For methods requiring higher throughput, shorter column lengths and higher flow rates can be used with minimal impact on resolution. For methods requiring maximum resolution, longer columns and shallower gradients can be investigated. Typical flow rates for columns are:

Column ID	Flow Rate
4.6 mm ID Columns	1.0-2.0 mL/min
2.0 mm ID Columns	0.25-0.5 mL/min

System Optimization

Aeris 3.6 µm core-shell columns operate comfortably with the pressure limits of conventional LC instruments and typically meet or exceed the performance of sub-2 µm particle and 3 µm fully porous particle columns on UHPLC systems. However, to maximize the benefits from your Aeris core-shell columns one should investigate:

- Minimizing sample dispersion before the column by reducing the system and dwell volume between the mixer and column inlet:
    - Minimize the length of all connecting tubing and reduce the ID of tubing wherever possible
    - Use 0.12 mm ID (0.005 in.) tubing whenever possible, avoid 0.25 mm ID (0.010 in.) throughout the HPLC system.
    - Use precut SS tubing or inspect every PEEK connection to assure that every tubing end is a square cut using a PEEK Tubing Cutter (AT0-1110). Improperly cut tubing WILL result in significant loss of system performance.
    - Use zero dead-volume fittings and ensure that all tubing is seated properly at every connection



- Minimizing peak dispersion after the column by reducing the system volume between the column and the detector:
    - Reduce tubing volume as described above
    - Bypass column switching apparatus whenever possible
    - Use a low volume flow cell whenever possible if UV detection is being used (for best results replace standard flow cells with <3 µL flow cells)
    - If MS detection is being used, bypass the UV detector to reduce extra column volume contribution from the flow cell. Investigate low volume electrospray interface solutions.

Optimize detector setting by adjusting the scan rate and/or time constant to the fastest practical setting such that signal-to-noise (s/n) is not adversely effected.

NOTE:
For additional assistance in optimizing your HPLC system contact your Phenomenex representative or look online at:

WWW.PHENOMENEX.COM/AERIS

OPTIMIZING YOUR METHOD ON AERIS WIDEPORE

To decrease runtime and sharpen peaks, use a shorter column, increase temperature, or run at a higher flow rate for equilibration and wash steps.

Using a shorter column will cut the runtimes down proportional to the decrease in length. You may lose some resolution between critical components which should be considered.

Increasing flow rates during the wash and equilibration steps can both increase sample throughput and improve column cleaning (leading to longer column lifetime). Due to the low operating backpressure of the column, flow rates during wash and equilibration can be increased significantly without exceeding operating limit of ones HPLC system.

Increasing column temperature generally reduces protein retention and sharpens protein peak shapes. Adding IPA to the acetonitrile organic mobile phase can further reduce protein retention and improve peak shape.

For increased resolving power use a longer column, shallow the gradient slope, and lower the initial percentage organic at the start of the gradient. Modifying the flow rate (higher or lower) can sometimes improve protein resolution.

Using different percentages of IPA or THF in the acetonitrile organic mobile phase can modify phase selectivity potentially improving resolution of critical components.

Investigate other Aeris phases for different selectivity (C4, XB-C8, or XB-C18).

To increase sensitivity, investigate using smaller ID Aeris WIDEPORE columns (i.e. 2.1 mm vs. 4.6 mm) which operate at a lower optimal flow rate. One needs to make sure that their system has been optimized for using smaller ID columns. Ideally, extra-column volume should be minimized to reduce sample or peak dispersion.

OPTIMIZING YOUR METHOD ON AERIS PEPTIDE

For increased resolving power, use a longer column, preferably a 250 mm (or 150 mm for the Aeris 1.7 µm XB-C18). Due to the lower backpressure of Aeris 3.6 µm, one can easily run 250 mm columns on both HPLC and UHPLC systems, AND one can couple multiple 250 mm columns together and run them inline for even better results.

NOTE:
Aeris PEPTIDE 1.7 µm should not be coupled due to their higher operating column backpressure.

As mentioned with optimizing Aeris WIDEPORE columns, one can use shallower gradients, lower initial organic concentration, and vary the flow rate used to improve resolution. For specific peptide map applications one can also use different gradient slope segments to "stretch" or "compress" regions of a peptide map.

Especially for peptide maps where larger peptides are contained within the mixture, one can potentially investigate using the Aeris WIDEPORE column instead of the Aeris PEPTIDE column for alternate selectivity.

To increase sensitivity, investigate using smaller ID Aeris PEPTIDE columns (i.e. 2.1 mm vs. 4.6 mm) which operate at a lower optimal flow rate. One needs to make sure that their system has been optimized for using smaller ID columns. Ideally, extra-column volume should be minimized to reduce sample or peak dispersion.

To decrease runtime and sharpen peaks, use a shorter column, increase temperature, or run at a higher flow rate for equilibration and wash steps.

Using a shorter column will cut the runtimes down proportional to the decrease in length. You may lose some resolution between critical components which should be considered.

Increasing flow rates during the wash and equilibration steps for the Aeris PEPTIDE 3.6 µm column can increase sample throughput. Due to the low operating backpressure of the column, flow rates during wash and equilibration can be increased significantly without exceeding operating limit of ones HPLC system.

NOTE:
Operating backpressure limits of HPLC and UHPLC systems limits the amount one can increase flow rates for Aeris PEPTIDE 1.7 µm columns during wash and re-equilibration.

For operators using systems that are UHPLC pressure capable (600 bar or 1000 bar system) can potentially use Aeris 1.7 µm columns to reduce runtimes while maintaining high efficiency and resolution with shorter runtimes.

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AERIS COLUMN ORDERING INFORMATION

Aeris WIDEPORE 3.6 µm Minibore Columns (mm)

	50 x 2.1	100 x 2.1	150 x 2.1	250 x 2.1
XB-C18	00B-4482-AN	00D-4482-AN	00F-4482-AN	00G-4482-AN
XB-C8	00B-4481-AN	00D-4481-AN	00F-4481-AN	00G-4481-AN
C4	00B-4486-AN	00D-4486-AN	00F-4486-AN	00G-4486-AN

Aeris WIDEPORE 3.6 µm Analytical Columns (mm)

	100 x 4.6	150 x 4.6	250 x 4.6
XB-C18	00D-4482-E0	00F-4482-E0	00G-4482-E0
XB-C8	00D-4481-E0	00F-4481-E0	00G-4481-E0
C4	00D-4486-E0	00F-4486-E0	00G-4486-E0

Aeris PEPTIDE 1.7 µm Minibore Columns (mm)

	50 x 2.1	100 x 2.1	150 x 2.1
XB-C18	00B-4506-AN	00D-4506-AN	00F-4506-AN

Aeris PEPTIDE 3.6 µm Minibore Columns (mm)

	50 x 2.1	100 x 2.1	150 x 2.1	250 x 2.1
XB-C18	00B-4507-AN	00D-4507-AN	00F-4507-AN	00G-4507-AN

Aeris PEPTIDE 3.6 µm Analytical Columns (mm)

	100 x 4.6	150 x 4.6	250 x 4.6
XB-C18	00D-4507-E0	00F-4507-E0	00G-4507-E0

Fittings and Tools

Part No.	Description	Unit
AQ0-8503	Sure-Lok High Pressure PEEK 1-pc Nut 10-32, for 1/16 in. Tubing, 12,000 psi (827 bar)	10/pk
AQ0-8504	Sure-Lok High Pressure PEEK Nut, 10-32, for 1/16 in. Tubing, 19,000 psi (1310 bar) for use with AQ0-8505	10/pk
AQ0-8505	Sure-Lok PEEK/Metal Ferrule Assembly 2-pc, for use with High Pressure Nut (AQ0-8504)	10/pk
AQ0-8506	Nut And Ferrule Set, SS, 10-32, for 1/16 in. Tubing, 28,000 psi (1930 bar)	10/pk
AQ0-8530	Sure-Lok Fitting Tightening Tool, Aluminum	ea
AT0-1110	PEEK Cutting Tool	ea

Sure-Lok Fitting Tightening Tool is required for AQ0-8503 and AQ0-8504.

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