Agilent Bio IEX Columns

Data Sheet

Agilent Bio IEX columns are packed with polymeric, nonporous, ion-exchange particles, and are designed for high resolution, high recovery, and highly efficient separations of proteins, peptides, oligonucleotides, and other biomolecules. The highly crosslinked and rigid nonporous poly(styrene divinylbenzene) (PS/DVB) particles are grafted with a hydrophilic, polymeric layer, virtually eliminating non-specific binding, while increasing efficiency and recoveries. Highly uniform and densely packed ion-exchange functional groups are chemically bonded to the hydrophilic layer. The Agilent Bio IEX family offers strong cation exchange (SCX), weak cation exchange (WCX), strong anion exchange (SAX), and weak anion exchange (WAX) phases. All phases are available in 1.7, 3, 5, and 10-µm nonporous particle sizes. The 10 and 5-µm particles are available in PEEK and stainless steel hardware. The 3 and 1.7-µm particles are available in stainless steel only.

Basic characteristics

Column phases	SCX (strong cation exchange) WCX (weak cation exchange) SAX (strong anion exchange) WAX (weak anion exchange)
Packing	Nonporous, poly(styrene divinylbenzene) (PS/DVB), grafted hydrophilic coating and bonded with a uniform, ion exchange layer
Particle size	1.7, 3, 5, and 10 µm
Pore structure	Nonporous
pH stability	2–12
Operating temperature limit	80 °C
Recommended maximum operating pressure limit	275 bar (~4,000 psi) for 10-µm particles 400 bar (~6,000 psi) for 5-µm particles 400 bar (~6,000 psi) for 3-µm particles 400 bar (~6,000 psi) for 1.7-µm particles
Mobile phase compatibility	Compatible with aqueous solution buffers, acetonitrile/acetone/methanol/ethanol, and water mixtures. Commonly used buffers: phosphate, Tris, MES, and acetate
Working flow rate	Typical range is 0.3 to 1.0 mL/min for a 4.6-mm column or 0.1 to 0.5 mL/min for a 2.1-mm column.

Note: To prevent sudden pressure increase, always start with a low flow rate and default to the maximum particle pressures. Gradually increase the flow rate to the designed operating flow rate condition and allow the baseline to flatten.

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Installing the column

Before installing the column, remove both endcaps and ensure that the flow direction matches the arrow on the column. Reverse flow is only used for column cleaning to remove material blocking the inlet.

Ensure tight ferrule connections before applying flow to the column. The recommended tubing is 1/16 in od PEEK or stainless with standard HPLC PEEK or stainless ferrules and nuts. When using 1.7-µm or 3-µm particle columns, ensure that connections are very tight, as the column backpressure will be higher than with larger particle columns.

Buffers and samples

Before use, filter all buffers and, if possible, samples through a 0.2-µm or 0.45-µm filter. This will prevent column clogging. Agilent Bio IEX columns are compatible with commonly used aqueous buffers and those containing a small amount of organic solvent. Water and organic mixtures, such as acetonitrile, methanol, and ethanol can also be used.

SCX and WCX columns are compatible with small amounts of nonionic and zwitterionic detergents, but are NOT compatible with cationic detergents.

SAX and WAX are compatible with samples containing small amounts of nonionic and zwitterionic detergents, but are NOT compatible with anionic detergents.

Note: samples are best dissolved in mobile phase A (low salt concentration buffer).

Column equilibration

Agilent Bio IEX columns are shipped in a 20 mM phosphate buffer, pH 6.0. Before the first sample injection, at a low flow rate (50 % of the operating flow rate), purge the column with mobile phase B (or a high salt concentration mobile phase) for 10 column volumes (CV), and equilibrate the column with mobile phase A until the baseline is stable. Gradually, increase the flow rate to the designed operating flow rate condition. Once the column is equilibrated, the column is ready for sample injection. Keep in mind that equilibration time is also needed after each run when using salt, pH, or combination gradients.

Column cleanup

An increase in column backpressure is likely to occur over time. Absorption of protein to the packing material or on the inlet frit will cause this increase in pressure, and will decrease column performance. Cleaning the column can decrease the backpressure and improve performance. To clean the column, flush the column in the reverse direction for at least 15 CV at no more than 50 % of the maximum operating pressure limit of the particle size.

Mild contamination

Columns can be efficiently cleaned with 1.0 M NaCl in mobile phase A solution (equilibration buffer).

Moderate contamination

For SCX and WCX columns
The recommended cleaning buffer for the SCX and WCX columns is 50 mM phosphate buffer in 1.0 M NaCl, pH 10.

For SAX and WAX
The recommended cleaning buffer for the SAX and WAX columns is 150 mM potassium nitrate, pH 4.0.

Severe contamination

Columns can be washed with: 0.1M NaOH for 15–20 CV at 0.25 mL/min. Increase the column temperature to 40 °C during the wash. After that, rinse the columns with at least 10 CV deniozed water. Next, rinse the columns with 0.1 M HCl for 15–20 CV. Wash the columns with 10 CV deniozed water. Next, the columns can be flushed with 5 CV of 100 % mobile phase B. Then, equilibrate the columns with equilibration buffer (such as mobile phase A or a gradient mixture of mobile A and B) for 10 CV.

Remove hydrophobic contaminants

If the columns are contaminated with hydrophobic proteins, these can be removed using 50–75 % ethanol or acetonitrile for at least 15 column volumes. After this step, the columns should be rinsed with 100 % deinozed water for 10 CV to remove organic solvents. Then, the columns can be equilibrated with mobile phase A or a gradient mixture of mobile A and B for 10 CV before the first injection.

Before using organics to clean the columns, ensure that the columns are flushed with water to remove most of the salt. This will prevent salt precipitation, which could increase column backpressure significantly and damage the columns.

Column storage

Short term storage

For short term column storage, flush the columns with mobile phase A at least 15 CV. Disconnect the columns from the LC system, and tightly seal both ends with the removable end plugs provided with the column. The column can be refrigerated or stored at room temperature (4 to 30 °C).

Extended storage

For extended column storage, flush the columns with 50 mM NaOH for at least 20 CV at 0.25 mL/min. Disconnect the columns from the LC system, and tightly seal both ends with the removable end plugs provided with the column. The column can be refrigerated or stored at room temperature (4 to 30 °C).

After long term storage, the column should be washed with water 10–20 CV to remove NaOH at 50 % reduced flow rate. After that, the column should be washed with 100 % mobile phase that contains high salt, such as 500 mM NaCl. Then, the column can be equilibrated with 10 to 20 mM binding mobile phase.

Use 20 % ethanol in mobile phase A for both short and extended column storage. Flush the column with at least 20 CV at 0.25 mL/min. Disconnect the columns from the LC system, and tightly seal both ends with the removable end plugs provided with the column. The column can be refrigerated or stored at room temperature (4 to 30 °C).

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Printed in the USA
March 27, 2017
5991-6120EN

Agilent Technologies