Agilent Bio MAb Columns

Data Sheet

About Agilent Bio MAb Columns

Agilent Bio MAb columns are packed with polymeric, nonporous, weak cation exchange particles, and are designed for high resolution, high recovery and highly efficient separations of monoclonal antibodies. The highly cross-linked and rigid nonporous poly(styrene divinylbenzene) (PS/DVB) particles are grafted with a hydrophilic, polymeric layer, virtually eliminating nonspecific binding of antibody proteins, increasing efficiency and recoveries. A highly uniform, densely packed, weak cation exchanger (carboxylate functional group) is chemically bonded to the hydrophilic layer. The Agilent Bio MAb family offers columns packed with 1.7, 3, 5 and 10 µm nonporous particles. 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.

Safety Precautions

Agilent Bio MAb columns are designed for use with high pressure liquid chromatography (HPLC) systems. Loose fittings and connections can cause buffer leaks and sample loss. Many samples and buffers are considered hazardous and should be treated as such. Always wear gloves and safety glasses when using columns in an HPLC system . Agilent does not recommend opening HPLC columns for any reason.

Installing the Column

Before installing the column, remove both endcaps and ensure that your flow direction matches the arrow on the column. Use the recommended flow direction unless reverse flow is being used to remove material blocking the inlet. Prior to applying flow over the column, make tight ferrule connections. The recommended tubing is 1/16" od PEEK or stainless tubing with standard HPLC PEEK or stainless ferules and nuts. When using 1.7 µm or 3 µm particle columns, ensure you have very tight connections as the column back pressure will be higher than with larger particle columns.

Basic Characteristics

Column Phase	Weak Cation Exchange (carboxylate)
Packing	Nonporous, poly(styrene divinylbenzene) (PS/DVB), grafted hydrophilic coating and bonded with a uniform, weak cation exchange layer
Particle size	1.7, 3, 5 and 10 µm
Pore structure	Nonporous
pH stability	2–12
Operating temperature limit	80 °C
Column hardware operating pressure limit	600 bar (8,700 psi) for stainless steel column hardware 400 bar (5,800 psi) for PEEK column hardware
Particle operating pressure limit	275 bar (4,000 psi) for 10 µm particles 413 bar (6,000 psi) for 5 µm particles 551 bar (8,000 psi) for 3 µm particles 689 bar (10,000 psi) for 1.7 µm particles
Mobile phase compatibility	Compatible with aqueous solution buffers, acetonitrile/acetone/methanol and water mixtures. Commonly used buffers: phosphate, tris, MES and acetate
Working flow rate	Typical range is 0.1-1.0 mL/min for a 4.6 mm or 2.1 mm id column, always start with a low flow rate and default to the maximum hardware and particle pressures

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Buffers and Samples

Prior to use, filter and degas all buffers and if possible samples through a 0.2 µm or 0.45 µm filter, this will prevent column clogging and air bubbles in the buffers. The use of a 0.5 µm frit filter or an Agilent Bio MAb guard can further protect the column. Bio MAb columns are compatible with commonly used aqueous buffers including: phosphate, potassium, sodium, sodium chloride, acetate, Tris and MES-containing buffers. Water and organic mixtures, such as acetonitrile and methanol can also be used. The use of an inline degassor is recommended; or at minimum the buffers should be degassed by filtration or sonicated under water-pumped vacuum. Agilent Bio MAb columns are compatible with nonionic and zwitterionic detergents, but are NOT compatible with cationic detergents.

Column Equilibration

Agilent Bio MAb columns are shipped in 20 mM phosphate buffer, pH 6.0. Prior to the first sample injection, purge the column with 20 column volumes of the loading buffer (Buffer A) at 0.1 ml/min. Gradually increase the flow rate until you reach your intended operating conditions and allow the baseline to flatten.

If the baseline or column back pressure fluctuates, increase the flow for 3-5 minutes, keeping in mind the maximum pressure for each particle size. Once equilibrated and the baseline is flat, the column is ready for a sample injection. Keep in mind that equilibration time is needed after each run when using salt, pH or combination gradients.

pH Stability

Agilent Bio MAb columns can be used in the range pH 2–12. Using the column within this range will optimize performance and lifetime.

Column Hardware and Particle Pressure

Agilent Bio MAb stainless steel column hardware has a maximum operating pressure of 600 bar (8,700 psi). PEEK hardware has a maximum operating pressure of 400 bar. The Agilent Bio MAb particles can withstand the following maximum pressures: 275 bar (4,000 psi) for the 10 µm particles, 413 bar (6,000 psi) for the 5 µm particles, 551 bar (8,000 psi) for the 3 µm particles and 689 bar (10,000 psi) for the 1.7 µm particles. Long term use at high flow rates may damage or decrease the lifetime of the column. Column back pressure commonly increases over the lifetime of the column. If there is a sudden increase in column back pressure it may be due to a clogged inlet frit. Reversing the flow to flush the column may clear the clogged inlet frit.

Temperature

The maximum column operating temperature is 80 °C. Long term use at 80 °C or higher will damage the column, especially when being used outside of the recommended pH range, <2 or >12. Column lifetime is optimized when used between 10–50°C.

Flow Rate Range

Normal operation is 0.1–1.0 mL/min for 4.6 mm or 2.1 mm id columns. When optimizing the flow rate, always default to the maximum column hardware and particle pressures.

Guard and Column Cleanup

An increase in guard or column back pressure 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 guard and/or column may decrease the back pressure and improve performance. When using a guard column or precolumn filter, remove the main column and flush the guard/filter in the reverse flow direction with 50 mM phosphate buffer, 1M NaCl, pH 10, for at least 15 minutes or replace the guard/filter during your next column use. To clean the main column, flush the column in the reverse direction with 50 mM phosphate buffer, 1M NaCl, pH 10 for at least 15 column volumes at no more than 50% of the maximum particle pressure limit. For basic proteins, flush the column with a low pH salt cleaning buffer. For acidic proteins, flush the column with a higher pH salt containing cleaning buffer. Hydrophobic proteins can be removed using an organic containing cleaning buffer.

Extended Storage

For extended column storage, flush the Agilent Bio MAb columns using 20 mM phosphate buffer with 0.1% NaN_3 (sodium azide) at pH 6.0. Tightly seal both ends with the removable end plugs provided with the column. The column can be refrigerated or stored at room temperature (4–35 °C).

Column Protection

Guard columns can be used for added column protection and are recommended. Guards and filters often capture particulates coming from the samples, the buffers, or from the HPLC system being used.

Agilent Ordering Information

For more information on our products, visit our Agilent Technologies home page on the World Wide Web at: http://www.agilent.com/chem/supplies For Technical support in the US and Canada, call 1-800-227-9770 or call your local Agilent sales office.

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