

Agilent Bio SEC-5 LC Columns



About Agilent Bio SEC-5 LC columns

Agilent Bio SEC-5 columns are packed with 5 μm silica particles coated with a proprietary neutral, hydrophilic layer. The thin polymeric layer is chemically bonded to highly pure and mechanically stable silica in an extremely controlled process. This uniform surface layer provides a highly efficient and stable size exclusion particle. Agilent Bio SEC-5 columns are available in 5 μm particles with 100 \AA , 150 \AA , 300 \AA , 500 \AA , 1,000 \AA , and 2,000 \AA nominal pore sizes. The highly spherical and narrowly dispersed 5 μm particles are packed using a proprietary technique, ensuring a uniform and stable packed bed. The Agilent Bio SEC-5 columns offer superior column lifetime and reproducibility. Agilent Bio SEC-5 columns are designed for a broad range of size-based, biomolecule separations, including antibodies, proteins, peptides, oligonucleotides, polysaccharides, polymers, biological cells, bacteria, viral particles, and other large biomolecules. Agilent Bio SEC-5 columns are best used with most aqueous buffers and have excellent stability in high and low salt conditions.

Basic characteristics

Column Phase	Size exclusion
Packing	Spherical, high purity, porous silica with a hydrophilic polymeric coating
Particle Size	5 μm
Pore Structure	100 \AA , 150 \AA , 300 \AA , 500 \AA , 1,000 \AA , 2,000 \AA
Column Exclusion Limits (in Daltons)	100 \AA MW range: 100 to ~100,000 150 \AA MW range: 500 to ~150,000 300 \AA MW range: 5,000 to ~1,250,000 500 \AA MW range: 15,000 to ~5,000,000 1,000 \AA MW range: 50,000 to ~7,500,000 2,000 \AA MW range: >10,000,000
pH Stability	2 to 8.5
Operating Temperature Limit	Recommended range: 10 to 30 $^{\circ}\text{C}$, maximum: 80 $^{\circ}\text{C}$
Operating Pressure Limit	Recommended operating pressure: 137 bar (2,000 psi) Maximum pressure: 240 bar (3,500 psi)
Mobile Phase Compatibility	Recommended: 150 mM phosphate buffer, pH 7.0, other aqueous buffers with high and low salt can be used
Working Flow Rate	0.1 to 1.25 mL/min for 7.8 mm id columns 0.1 to 0.4 mL/min for 4.6 mm id columns

Safety precautions

Agilent Bio SEC-5 columns are designed for use with high-performance 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. Before applying flow over the column, make tight ferrule connections. The recommended tubing is 1/16 inch od PEEK or stainless steel tubing with standard HPLC PEEK or stainless steel ferrules and nuts.

Buffers and samples

Before use, filter and degas all buffers and, if possible, samples through a 0.2 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 SEC-5 guard can further protect the column. Agilent Bio SEC-5 columns are compatible with commonly used aqueous buffers. Water and organic mixtures, such as acetonitrile and methanol can also be used. The use of an inline degasser is recommended; or at minimum the buffers should be degassed by filtration or sonicated under water-pumped vacuum.

Column equilibration

Agilent Bio SEC-5 columns are shipped in 0.05% azide in 0.1 M sodium phosphate buffer, pH 7.0. Before the first sample injection, purge the column with 20 column volumes of 150 mM sodium phosphate buffer, pH 7.0 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 backpressure fluctuates, increase the flow for 3 to 5 minutes, keeping in mind the maximum particle pressure of 240 bar (3,500 psi). 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.

pH stability

Agilent Bio SEC-5 columns can be used in the range pH 2 to 8.5. Using the column within this range will optimize performance and lifetime.

Column hardware and particle pressure

The recommended operating pressure for both the 7.8 and 4.6 mm id columns is 137 bar (2,000 psi). Column lifetime is increased when operating at or below this pressure. Do not exceed the maximum particle pressure of 240 bar (3,500 psi). Long term use at high flow rates may damage or decrease the lifetime of the column. Column backpressure commonly increases over the lifetime of the column. If there is a sudden increase in column backpressure, this may be due to a clogged inlet frit. Reversing the flow to flush the column may clear the clogged inlet frit.

Temperature

Column lifetime is optimized when used between 10 to 30 °C. The maximum column operating temperature is 80 °C. Long term use at 80 °C or higher will damage the column, especially when being used at above pH 8.

Flow rate range

The working flow rate is 0.1 to 1.25 mL/min for the 7.8 mm id columns and 0.1 to 0.4 mL/min for 4.6 mm id columns.

Guard and column cleanup

An increase in guard or 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 guard or column may decrease the backpressure 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 cleaning buffer 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 cleaning buffer for at least 15 column volumes at no more than 50% of the maximum particle pressure limit. Rinse well with 3 to 5 column volumes of Milli-Q or Nanopure water after each cleaning solution and before reconditioning the column for use. Two cleaning buffers are recommended.

- Concentrated neutral salts, such as 0.5 M Na₂SO₄, pH 3.0
- Aqueous buffer with water soluble organic, such as 50 mM phosphate, pH 7.0 with 10 to 20% methanol, acetonitrile, or ethanol

Note: When using 10 to 20% methanol, acetonitrile or ethanol, a low starting flow rate of 0.1 mL/min is recommended which can be gradually increased to higher flow rates if the pressure is kept below 240 bar.

Extended storage

For extended column storage, flush the column in a 0.05% NaN₃ (sodium azide) or 20% ethanol containing buffer at pH 7.0 for at least 10 column volumes and tightly seal both ends with the removable end plugs provided with the column. Before introducing 20% ethanol or methanol for storage, ensure that all salts are flushed from the column. A low starting flow rate at 0.1 mL/min is recommended. The flow rate can be gradually increased to higher flow rates if the pressure is kept below 240 bar. The column can be refrigerated or stored at room temperature (4 to 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

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