User Guide

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Agilent BioHPLC and AdvanceBio Columns

This guide provides general information for the Agilent BioHPLC and AdvanceBio ion-exchange columns. For additional detailed information about your specific phase or family, visit: agilent.com/chem/biocolumnchoices.

Getting started

A QC Column Performance Report, including a test chromatogram, is enclosed with every Agilent column. The QC test system has been modified from a standard system to minimize system dead volume, so it may vary from the system used in your lab. This allows a better evaluation of the column efficiency and assures a more consistent product. An optimized LC system will generate similar results to the chromatogram on your QC Performance Report.

If you have specific questions, contact the Technical Support team at agilent.com/chem/columnsupport.

Using the column

Installation

- The direction of flow is marked on the column.
- Agilent recommends using Polyketone fittings (part number 5042-8957) for columns up to 600 bar, and 1,200 bar removable fittings (part number 5067-4733) for columns that will be operated at UHPLC pressures.

Polyketone fitting
(part number 5042-8957)

Agilent 1200 bar removable fitting
(part number 5067-4733)

Column conditioning

Every column is tested before shipment. For first use, the shipping solvent must be replaced with eluent, and the column conditioned with the correct counter ion. Take care that all components are miscible and soluble. Care should be taken to make sure the column has been properly equilibrated prior to use and before the start of each analysis in a sequence (see Table 1). This will ensure reproducibility and help prevent retention time drifting. When installing a column it is advisable to always start at a low flow rate, and as the pressure stabilizes, increase the flow rate to the operating flow rate that will be used for the analysis.

Table 1. Instructions before first use.

Column	Procedure
Bio IEX and Bio MAb: Flush out the shipping solution (20 mM phosphate buffer, pH 6.0) and condition with the required counter ion prior to use.	1. Purge the column with 20 column volumes of the loading buffer (buffer A) at 0.1 mL/min. Gradually increase the flow rate until intended operating conditions are reached; allow the baseline to flatten. 2. If the baseline or column backpressure fluctuates, increase the flow for 3 to 5 min, keeping in mind the maximum pressure for each particle size. 3. Once the column is equilibrated and the baseline is stable, the column is ready for a sample injection.
PL-SAX and PL-SCX: Flush out the shipping solution (0.1 M Na_2SO_4 and 0.02% sodium azide) and condition with the required counter ion prior to use. The following procedure is recommended at 0.5 mL/min for a 4.6 mm column.	1. Elute for five column volumes with the low ionic strength component of the mobile phase, buffer A. 2. Exchange the counter ion by eluting with the high ionic strength component of the mobile phase, buffer B. Continue with this eluent until a stable baseline is achieved at the required sensitivity, a minimum of five column volumes. 3. Equilibrate with buffer A for a minimum of five column volumes prior to use.
Bio-Monolith: Flush out the shipping solution (20% ethanol) and condition with the required counter ion prior to use.	1. Wash the column with at least 10 column volumes of the binding mobile phase (low ionic strength) at half of the working flow rate. 2. Wash the column with at least 10 column volumes of the high ionic strength buffer (e.g., buffer with 1.0 or 2.0 M NaCl) at one-half of the working flow rate. 3. Equilibrate with at least 10 column volumes (20 column volumes for the weak anion-exchange, DEAE, column) binding mobile phase (low ionic strength) at a working flow rate.

Important safety considerations

- All points of connection in liquid chromatographic systems are potential sources of leaks. Users should be aware of the toxicity or flammability of their mobile phases.
- Because of the small particle size, dry column packings are respirable. Agilent does not recommend removing the column end fittings and exposing the media. Columns should only be opened by trained personnel in a well-ventilated area.
- Please adhere to operating pressure limits noted for each column (see Table 2). Exceeding these limits will compromise chromatographic performance and column lifetime, and could be unsafe.

Table 2. Maximum operating parameters for columns up to 4.6 mm.

Column	pH Stability	Maximum Operating Temperature	Maximum Operating Pressure
Bio MAb	2.0 to 12.0	80 °C	1.7 µm = 689 bar 3 µm = 551 bar 5 µm = 413 bar 10 µm = 275 bar
Bio IEX (SCX, WCX, SAX, WAX)	2.0 to 12.0	80 °C	1.7 µm = 689 bar 3 µm = 551 bar 5 µm = 413 bar 10 µm = 275 bar
PL-SAX, PL-SCX	1.0 to 14.0	80 °C	207 bar
Bio-Monolith (QA, DEAE, SO3)	2.0 to 13.0	40 °C	150 bar

Other operating tips

- While generally not harmful to the column, reverse flow should be avoided except to attempt removal of clogged frit (see "column care").
- It is recommended that the flow rate is started at a reduced rate and then gently increased to the desired operating flow rate.
- Always use high purity reagents and chromatography grade solvent to prepare your mobile phase. Degas and filter all mobile phase prior to use.
- Disassembling a column will degrade column performance.
- If the column is used outside of recommended pH ranges for column phase (see Table 2), a reduced lifetime will result.
- An inline filter or guard column may be used to protect your column and increase its lifetime.
- Columns should not be maintained at elevated pH or elevated temperature when not in use.
- New columns may contain a mixture of organic solvents and water, which may contain buffer salts (see Table 3). Initially, care should be taken not to pass any mobile phase through the column that may cause a precipitate to form or may not be fully miscible.

Table 3. Shipping solvents.

Column	Shipping Solvents	Compatibility
Cation-exchange Agilent Bio WCX, Bio SCX, and Bio MAb	20 mM phosphate buffer, pH 6.0	All commonly used ion-exchange eluents, buffers and salts.
Agilent PL-SCX	0.1 M Na_2SO_4 and 0.02% sodium azide	Compatible with nonionic and zwitterionic detergents, but NOT with cationic detergents.
Anion-exchange Agilent Bio SAX and Bio WAX	20 mM phosphate buffer, pH 6.0	All commonly used ion-exchange eluents, buffers, and salts.
Agilent PL-SAX	0.1 M Na_2SO_4 and 0.02% sodium azide	Compatible with nonionic and zwitterionic detergents, but NOT with anionic detergents.
Agilent Bio-Monolith QA, DEAE, SO_3	20% Ethanol	All commonly used ion-exchange eluents, buffers, and salts. Up to 2.0 M NaOH and 1.0 M HCl. Aqueous solutions of up to 30% 2-propanol, methanol, or acetonitrile. Up to 50% acetic acid. Up to 70% ethanol. Enzymatic solutions such as pepsin, trypsin, and DNase.

Mobile phase selection and operating temperatures

Ion-exchange uses aqueous buffers to control pH, most commonly salt gradients for elution. Alternatively, a pH gradient can be used at constant ionic strength. The starting buffer pH should be at least one pH unit away from the isoelectric point for proteins and peptides.

Column care

Columns with a particle size of less than 2 µm have a 0.5 µm inlet frit. Particulates will block the column inlet frits and so should be removed before the sample is analyzed. Where this is not possible, an inline filter should be used to protect and increase the lifetime of the analytical column. It is recommended that samples are filtered before injecting them onto any column.

Cleaning your column to extend column lifetime

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 may decrease the backpressure and improve performance.

When using a guard column or precolumn filter, replace the guard or filter and remove the main column. To clean the column, flush the column in the reverse direction with the cleaning buffer for at least 15 column volumes at no more than 50% of the maximum particle pressure limit.

Table 4. Cleaning instructions.

Column	Column Cleanup
Bio SCX, Bio WCX, and Bio MAb	50 mM Phosphate buffer, 1 M NaCl, pH 10. 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.
Bio SAX and Bio WAX	150 mM Potassium nitrate in 75% acetonitrile, pH 2 (HCl adjusted).
PL-SAX and PL-SCX	1 M acid, e.g. acetic and 1 M base, e.g. sodium hydroxide. If contamination is due to small hydrophobic molecules, e.g. fats, detergents and peptides, then the matrix should be washed with an organic alcohol. The addition of 0.1% trifluoroacetic acid to the organic may be advantageous. After each washing sequence a high salt elution should be carried out, and after thorough cleaning, the column should be conditioned as per instructions (see Table 1).
Bio-Monolith (QA, DEAE, SO_3)	Wash with at least 2 mL of a buffer containing 1 M NaCl at 0.5 to 1.0 mL/min. Recondition the column with 5 to 6 mL of the starting mobile phase at 1 mL/min. For best results, repeat these steps at the end of each chromatographic run.

Storage recommendations

Long-term storage of ion-exchange columns can be done safely (see Table 5 for phase-specific instructions). Before storing, end-fittings should be tightly capped with end-plugs to prevent packing from drying out. Columns may be safely stored for one to several days in most mobile phases.

Table 5. Storage instructions.

Column	Flushing Instructions Before Long-Term Storage
Bio SCX, Bio WCX, and Bio MAb	Flush using 20 mM phosphate buffer (the shipping solution), with 0.1% NaN_3 (sodium azide) at pH 6.0. Flush for at least 15 column volumes.
Bio SAX and Bio WAX	Flush using 20 mM Tris with 0.1% NaN_3 (sodium azide) at pH 8.0. Flush for at least 15 column volumes.
PL-SAX and PL-SCX	Wash with 1 M sodium chloride. After flushing with water, the storage buffer of 0.1 M Na_2SO_4 containing 0.02% sodium azide can be introduced.
Bio-Monolith (QA, DEAE, SO_3)	If the column will not be in use for more than two days, it should be washed with at least 1 mL of DI water and afterwards flushed with at least 2 mL of a 20% ethanol solution at the flow rate of 0.2 to 0.5 mL/min, sealed with column end-plugs, and stored appropriately at 4 to 30 °C.

Tips for getting the best chromatographic results

- Optimize your instrument by minimizing tubing lengths between components to reduce extra column volume and band broadening. Use 0.12 mm id red tubing for Fast LC/high efficiency columns. Learn about capillary options at agilent.com/chem/lccapillaries.
- Ensure the data collection rate is optimized for your column.
- Use sample filtration or other sample prep as appropriate for your sample. Learn more about sample prep at agilent.com/chem/sampleprep.
- Use certified lamps in your LC instruments for best performance.

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