Data Sheet

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Agilent PL-SCX for Biomolecules Columns and Media

About Agilent PL-SCX for biomolecules

The PL-SCX (strong cation exchange) media has an optimized pore size and structure for the analysis of biological macromolecules. PL-SCX media is available in prepacked columns from 2.1 to 100 mm id or as bulk media up to 1 kg. 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.

For specific questions, contact the Technical Support team at agilent.com/chem/columnsupport for more information.

Basic characteristics

Parameter	Value
Column Phase	Cation exchange
Packing	Spherical, polymeric media
Particle Size	5, 8, 10, 30 µm
Pore Structure	Totally porous, 1,000 Å
pH Stability	1 to 14
Operating Temperature Limit	80 °C
Operating Pressure Limit	5, 8, 10 µm (207 bar) 30 µm (103 bar)
Mobile Phase Compatibility	All commonly used ion exchange eluents, buffers, and salts. Compatible with nonionic and zwitterionic detergents, but not compatible with cationic detergents.
Linear Flow Rate	180 to 360 cm/hr

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 of 207 bar for 5 to 10 µm, and 103 bar for 30 µm particles. Exceeding these limits will compromise chromatographic performance and column lifetime and could be unsafe.

Installation

Ensure that your LC instrument is configured correctly to minimize extracolumn band broadening, but to also ensure that there are no pressure restrictions that could lead to excessive operating pressure. Agilent recommends choosing capillary tubing of the appropriate internal diameter (id); 1/16 in stainless steel tubing is recommended for column connections.

Table 1. Recommended capillary inner diameter

1.0 to 2.0 mL/min	4.0 to 8.0 mL/min	15 to 40 mL/min	40 to 80 mL/min	80 to 200 mL/min
0.17 mm id	0.3 mm id	0.5 mm id	0.6 mm id	0.94 mm id

For more information on capillaries, please see HPLC Capillaries | Agilent

Before connecting your column, use a barrel connector and determine the backpressure from your LC system. Identify any causes of high backpressure and rectify any problems before installing your column.

Note: Agilent 50 and 100 mm id PL-SCX preparative columns are suitable for use with 1/8 in outer diameter (od) tubing using Valco 1/8 in nuts (PL1310-0038, 5/pk) and ferrules (PL1310-0038, 5/pk). Alternatively, 1/8 to 1/16 in reducers are supplied with each column to enable 1/16 in od tubing to be used where appropriate. The direction of flow is marked on your column.

Shipping eluent

PL-SCX columns are supplied containing 0.1 M Na_2SO_4 and 0.02% sodium azide. Columns are securely sealed with endcaps, which must always be replaced when the column is disconnected from the system to prevent columns from drying out.

Column compatibility

PL-SCX is compatible with all commonly used ion exchange eluents, buffers, and salts. PL-SCX is also compatible with nonionic and zwitterionic detergents, but is not compatible with cationic detergents.

Column conditioning

Wash out the shipping solution and condition with the required counter ion before use. The following procedure is recommended at 180 cm/h (0.5 mL/min for a 4.6 mm id column).

1. Elute for five column volumes with the low ionic strength component of the mobile phase buffer A. (e.g. 20 mM phosphate, pH 6.0).
2. Exchange the counter ion by eluting with the high ionic strength component of the mobile phase, buffer B (for example 20 mM phosphate, 0.5 M NaCl, pH 6.0). 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 before use.

Mobile phases

The PL-SCX media, being polymeric and macroporous, is stable in most polar mobile phases. The excellent chemical resistance of both the base polymer and of the cation exchange functionality enables the use of aqueous buffers in the pH range 1 to 14 without accelerated column degradation or loss of ionic capacity.

Both anionic and nonionic detergents can be used. However, the column will need conditioning with the required detergent before use. As a cation exchange column, cationic detergents should not be used.

The prepacked columns are stable in alcohols (C_1 to C_4). When changing mobile phases between alcohols and salt buffers, wash with at least five column volumes of H_2O.

Flow rate/pressure

The maximum operating pressure for the PL-SCX stainless steel HPLC column is 207 bar for 5 to 10 µm particles, and 103 bar for 30 µm particles. With low-viscosity mobile phases, linear flow rates of 180 to 360 cm/hr can be used.

Column id (mm)	Volumetric Flow Rate (mL/min)
4.6	0.5 to 1
7.5	1.3 to 2.7
25	14.7 to 29.5
50	58.8 to 117.8
100	235 to 471.7

If column pressures are high due to mobile phase viscosity or to improve sample solubility or resolution, elevated temperatures up to 80 °C can be used.

Sample preparation

The samples should be free from fat, which would otherwise contaminate the column, and be filtered (<0.5 µm). If turbid sample solutions are injected, even after being filtered, the lifetime of the column may be significantly reduced.

If possible, the samples should be dissolved in buffer A, the low ionic strength component of the mobile phase. For interaction to occur with the strong cation exchanger the solutes must be positively charged at the analysis pH. In the case of proteins where the total net charge is pH-dependent, this will be below the isoelectric point (pI) of the protein being analyzed. The pH can be controlled using any of the commonly used anionic buffers such as MES or phosphate buffers. The solutes can be eluted by increasing the ionic strength or changing the mobile phase pH.

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 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.

The excellent chemical stability of the PL-SCX media enables washing with 1 M acid (for example, acetic or hydrochloric acid) and 1 M base (for example, sodium hydroxide). If the contamination is due to small hydrophobic molecules (for example, fats or detergents) then the matrix should be washed with an organic alcohol such as 95% methanol. 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. After thorough cleaning, the column should be conditioned as detailed earlier.

Storage recommendations

When removing the column from the system, end-fittings should be tightly capped with end-plugs to prevent the packing from drying out. Columns may be safely stored for up to several days in most mobile phases.

For long-term storage, the column should be washed with 1 M sodium chloride. After flushing with water, the storage buffer of 0.1 M Na_2SO_4 containing 0.2% sodium azide can be introduced.

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DE67824093

Printed in the USA, May 19, 2022
5994-4894EN

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