Data Sheet

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Agilent PLRP-S for Biomolecules Columns and Media

Agilent PLRP-S for Biomolecules

The PLRP-S media has an optimized pore size and structure for the analysis and purification of biological macromolecules. PLRP-S 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	Reversed-phase
Packing	Spherical, polymeric media
Particle Size	3, 5, 8, 10, 10 to 15, 15 to 20, 30, and 50 µm
Pore Structure	Totally porous, 100 Å, 300 Å, 1,000 Å, and 4,000 Å
pH Stability	1 to 14
Operating Temperature Limit	200 °C
Operating Pressure Limit	3 µm (275 bar) 5, 8, 10 (207 bar) 10 to 15, 15 to 20, 30, 50 µm (103 bar)
Mobile Phase Compatibility	Aqueous organic solvents including N,N-dimethylformamide and dimethyl sulfoxide. 100% aqueous is not recommended, as it will reduce column performance and lifetime.
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 275 bar for 3 µm, 207 bar for 5 to 10 µm, and 103 bar for 15 to 50 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 and to 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, visit
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 PLRP-S 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

PLRP-S columns are supplied containing 7:1 acetonitrile:water. Columns are securely sealed with endcaps is disconnected from the system to prevent columns from drying out.

Column compatibility

PLRP-S is compatible with aqueous organic solvents including *n,n*-dimethylformamide and dimethyl sulfoxide. 100% aqueous is not recommended as it will reduce column performance and lifetime.

Column conditioning

Every column is tested before shipment. Before the first use, the shipping solvent must be replaced with eluent, taking care that all components are miscible and soluble. If mobile phase additives are used (such as buffers or ion-pair reagents), it is advisable to do an intermediate flush with a mobile phase of the correct composition, but without these additions. Flushing with 10 to 20 column volumes should help in transitioning to your mobile phase. Check that the column has been properly equilibrated before use. This will ensure reproducibility and help prevent retention time drifting.

When using formic acid as a mobile phase additive, condition the column as recommended in Table 2.

Table 2. Recommended formic acid mobile phase additive conditions.

Column id	Mobile Phase	Flow Rate (mL/min)	Column Temp. (°C)	Time (hrs)	After Conditioning
2.1 mm	95/5 H_2O/CH_3CN + 0.1% formic acid	0.1	60	4	Flush and store in 100% CH_3CN
4.6 mm	95/5 H_2O/CH_3CN + 0.1% formic acid	0.4	60	4	Flush and store in 100% CH_3CN

Mobile phases

The PLRP-S media, being polymeric and macroporous, is stable in most aqueous organic solvents including N,N-dimethylformamide and dimethyl sulfoxide. PLRP-S is best used with polar mobile phases, such as methanol/water or acetonitrile/water mixtures. Increasing the amount of organic component reduces the retention time of the sample. When the maximum temperature is used for prolonged periods, this will reduce column lifetime.

The excellent chemical resistance of both the base polymer enables the use of buffers in the pH range 1 to 14 without accelerated column degradation or loss of capacity.

Operating tips

- Reverse flow will not usually harm the column, but should be avoided except when removing a clogged frit.
- Start the flow rate at a reduced rate, and gently increase it to the desired operating flow rate.
- Always use high purity reagents and chromatography grade solvents to prepare your mobile phase. Degas and filter all mobile phase before use.
- Use an inline filter to protect your column and increase its lifetime.
- Avoid using 100% aqueous eluents with PLRP-S columns as they will significantly reduce the column lifetime and may result in a rapid deterioration in peak width and symmetry.

Flow rate/pressure

The maximum operating pressure for the PLRP-S stainless steel HPLC column is 275 bar for 3 µm, 207 bar for 5 to 10 µm particles, and 103 bar for 15 to 50 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 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. All eluents should be freshly prepared and filtered before use. If dealing with difficult samples prone to aggregation, inline filters are recommended to prevent column clogging.

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.

When performing cleaning, the column can be run in the reverse direction. Start with a stronger (less polar) solvent.

1. Disconnect the column from the detector and the run wash solvents into a beaker.
2. Start with the mobile phase without buffer salts (water/organic). Run 10 to 20 column volumes through.
3. Next, use 100% organic solvent (methanol or acetonitrile).
4. Check the pressure to see if it has returned to normal. If not, then:
5. Discard the column or consider stronger conditions, such as 75% acetonitrile:25% isopropanol.
6. Increase to 100% isopropanol, 100% methylene chloride, or 100% hexane (if you use methylene chloride or hexane, you will need to flush the column with isopropanol before use and before returning to your mobile phase as it is not miscible with aqueous).

With PLRP-S columns, additional aggressive cleanup cycles using 80% 1 M NaOH or 1 M HCl with 20% organic, can be used.

Storage recommendations

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

Where possible, flush the column and return it to the same storage solution in which the column was originally shipped. Do not leave columns stored in solutions that contain high concentrations of buffers or salts. If alternative storage solutions are required, ensure that there is no risk of bacterial growth occurring. Some storage solution combinations (such as aqueous alcohol) can be very viscous. To avoid pressure damage to the column, only use these storage solutions at a reduced flow rate.

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