Agilent ProSEC 300S Columns for Protein SEC

Data Sheet

ProSEC columns contain hydrophobic silica particles for SEC separations of biomolecules including proteins and peptides.

Installation

Stainless steel tubing of 1/16 in od and 0.010 in id or 0.007 in id is recommended for column connections. Connecting tubing lengths between columns, detectors and injection volumes should be minimized to avoid excessive dead volume which will diminish system performance. Column connections should be made using Parker compatible 1/16 in nuts and ferrules. The compatibility of column connectors is illustrated in Figure 1.

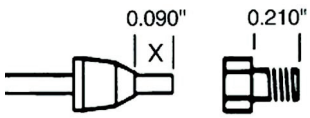

Figure 1. Compatible connectors.

The distance "x" for the standard ProSEC 300S column end fitting is 0.090 in and a minimum male nut length of 0.210 in is required. Some fittings for example, Waters, Valco and Rheodyne, are not compatible. If unsure, please contact Agilent for further advice.

Column connection

Connect the ProSEC 300S column in the eluent flow direction indicated and tighten the 1/16 in nut and ferrule using wrenches on the 1/16 in nut and the actual end fitting.

To avoid loosening the end fittings and causing leaks, wrenches must be used on the end fitting adjacent to the connecting nut and NOT on the column barrel or the opposite end fitting, as in Figure 2.

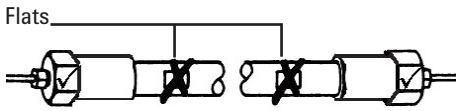

Figure 2. Don't use wrenches on the flats.

Eluent Selection and Flow Rate

ProSEC 300S columns are normally supplied in water containing 0.02% sodium azide and completely sealed with end caps that should always be in place when the column is not connected to a system. The recommendations and restrictions below must be followed.

- All eluents used should be of high purity and should be filtered and degassed before use.
- Buffered eluent systems within the pH range 5.0–7.5. Both high and low ionic strength may be used with no detrimental effect on the column.
- Organic solvents and organic/aqueous mixtures may be used with the ProSEC 300S column.
- When transferring from one eluent to another, the compatibility and solubility of any organic modifiers or salts/additives must be checked to prevent on-column precipitation which would irreversibly damage the column.
- The pressure across a column or series of columns should not exceed 3700 psi (250 bar).
- Elevating the temperature up to a maximum of 40 °C improves resolution.
- Flow rates should be changed progressively and pressure pulses limited.

The recommended flow rates are given in Table 1.

Table 1. Recommended Flow Rate

Column type	Typical flow rates (mL/min)	Recommended flow rate (mL/min)
ProSEC 300S 4.6 mm id	0.2–0.5	0.3
ProSEC 300S 7.5 mm id	0.5–1.5	1.0

Sample Preparation and Injection

If maximum resolution and expected column lifetime are to be achieved, care must be taken in sample preparation. To avoid blockage of the column frits, sample filtration is recommended (0.5–2.0 µm filter) and a guard column will protect the analytical columns with little effect on performance.

Optimum sample volumes and concentrations are best determined for each type of analysis and is dependent on sample MW. Broad distribution polymers can generally be injected at higher concentrations than lower polydispersity samples. Overloading will not damage the column, but distorted peaks and therefore spurious results will be obtained.

Excessive injector loop volume will contribute to band broadening and reduce system performance, particularly with high efficiency or narrow bore columns. Agilent's injection volume recommendations are shown in Table 2.

Table 2. Injection Volume Recommendations

Column type	Recommended concentration (%)	Recommended injection (µL) per column
ProSEC 300S 7.5 mm id	0.05–0.50	20–50
ProSEC 300S 4.6 mm id	0.01–0.20	1– 20

Column Testing

Every column is supplied with a test certificate indicating the column performance. This includes a protein separation and a measurement of column performance.

Column efficiency is dependent on many experimental factors (system dead volume, eluent, flow rate, test probe, temperature, and so forth) and test results may differ slightly from those quoted on the column certificate due to variability in these parameters. It is vital to ensure that the system dispersion is minimized in order to obtain the full potential of Agilent columns.

Table 3. Column Specifications

Specifications	ProSEC 300S
Typical operating pressure psi (bar)[1]	900 (60)
Maximum operating pressure psi (bar)	3700 (250)
Maximum operating temperature °C	40
Efficiency ppm[1]	> 75,000

[1] Based on H_2O at 20 °C ProSEC 300S, 300 × 7.5 mm at 1.0 mL/min using vitamin B12.

Storage

On removing the column from the system, the end plugs must be replaced to prevent the column from drying out by evaporation. End Plugs need only be finger tight. For long term storage, the column should be flushed with water and stored in water containing 0.02% sodium azide.

Maintenance

Deterioration in column performance may occur as a result of damage to the packed bed or as a result of blockage in the column frits. In the case of frit blockage, the column can be reverse flushed at 1.0 mL/min for 1 minute to remove loosely retained material.

For further technical assistance, please contact your local Agilent office.

Agilent Ordering Information

For more information on our products, visit our web site at www.agilent.com/chem/columns.

www.agilent.com/chem

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