

Care and Use for MONOBIO SEC Columns

Description

MONOBIO SEC is a family of high performance, size exclusion chromatography columns designed for separating a broad range of biomolecules based on size. This column technology involves the creation of a neutral hydrophilic layer on the surface of specially designed, high-strength, monodispersed silica particles, combined with well-established column packing processes. MONOBIO SEC columns are suited for separating peptides, oligonucleotides, proteins, monoclonal antibodies, plasmids, AAVs, and related aggregates and fragments, thus have a broad application range in pharmaceutical, biopharmaceutical, biotechnology and academia research.

Main Features

- High column efficiency and high resolution
- Minimal undesired interactions between the stationary phase and analytes, resulting in good peak shape and recovery
- High mechanical strength for extended column lifetime
- Broad range of applications, including small-molecule drugs, peptides, proteins, glycans, oligos, plasmids, AAVs, etc.

Product Information

Product Name	MONOBIO SEC
Functional Group	Diol
Substrate	Monodispersed, high pore volume, porous silica particles
Particle Size	1.8, 3 & 5 μm
Pore Size	120, 150, 300, 500, 700, 1000, 2000 $\text{\AA}$
Pressure Limit	6000 psi (1.8 μm) 2500 psi (3 μm) 1500 psi (5 μm)
Temperature Limit	40 $^{\circ}\text{C}$
pH Range	2-8
Flow Rate	0.1 - 0.4 mL/min (for 4.6 mm i.d. columns) 0.3 - 1.0 mL/min (for 7.8 mm i.d. columns)

Tips: To obtain optimal separation, minimize extra column volume in areas such as the connection tubing, detector flow cell and connections. When using a 4.6-mm i.d. column, a connection tubing with smaller than 0.13 mm i.d. and a semi-micro flow cell are often required to obtain satisfactory separation.

Column Care

- Operate the column within its specifications shown above (i.e., pressure, flow rate, pH, temperature).
- Flow should follow the direction of the arrow on column.
- Avoid physical shock to the column.
- Avoid pressure surge during use.
- Only use high quality HPLC solvents, water, buffer salts, or other additives.

- Always filter the mobile phase to prevent particulates from clogging the LC system or the column.
- Good sample clean-up before injection.
- In-line filter and/or guard column are strongly recommended for real-life samples.
- To ensure the quality of data, fully equilibrate the column with the mobile phase for at least 10 column volumes before injection.

Mobile Phase Preparation

Phosphate buffers are often used for SEC separations. To shield undesired interactions between the analyte of interest and stationary phase, a certain concentration of a salt (i.e., 300 mM NaCl) and/or an organic solvent (i.e., 10% acetonitrile) can be added to the mobile phase. Two suggested mobile phases with preparation are listed below:

50 mM phosphate buffer (pH6.8) containing 300 mM NaCl:

weigh 1000 ± 1 g D.I. water, then add 3.00 ± 0.05 g sodium dihydrogen phosphate (anhydrous), 3.55 ± 0.05 g disodium hydrogen phosphate (anhydrous) and 17.54 ± 0.05 g NaCl, mix well and filter through 0.2 μm membrane before use.

50 mM phosphate buffer (pH6.8) containing 300 mM

NaCl/acetonitrile (90:10, v/v): measure 900 ± 1 mL 50 mM phosphate buffer (pH 6.8) containing 300 mM NaCl, and 100 ± 0.5 mL acetonitrile, mix well and filter through 0.2 μm membrane before use.

Column Conditioning

When using a new column for the first time, a high concentration of the analyte of interest should be injected onto the column to “mask” any possible undesired binding sites before analysis to ensure the quality of data.

Column Cleaning

After certain time of use, if there is any indication of contamination in the column (i.e., peak broadening, tailing, or splitting, retention shift, change in resolution or backpressure increase), wash the column as follows:

Procedure:

To remove ionic species: wash the column with a high salt concentration solution (i.e., 0.25 M NaCl) or acidic phosphate buffer (i.e., pH2.5) for at least 10 column volumes, followed by washing with D.I. water for 10 column volumes.

To remove hydrophobic species: wash the column with approximately 15% organic solvent (i.e., methanol or acetonitrile) for at least 10 column volumes, followed by washing with D.I. water for 10 column volumes.

To remove hydrogen bonding species: wash the column with 6M urea (aq.) or 0.2% nonionic surfactants (i.e., Triton, Tween,

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Brij, etc.) for at least 10 column volumes, followed by washing with D.I. water for 10 column volumes.

Caution: Frequently exposing the column to different solutions may result in column damage, thus take extra precautions when conducting column cleaning.

Tips: During column cleaning, disconnect the flow cell from the column outlet to prevent contamination to the LC system.

Tips: During column cleaning, use ~10-50% of normal flow rate to avoid pressure surge.

Tips: When high backpressure is the issue, back flush the column using above procedure.

Column Storage

Short-term storage: Mobile phase

Long-term storage: 20% ethanol, methanol or acetonitrile; or 0.05% sodium azide (aq.)

Caution: Make sure that all buffers are washed out of the column before flushing with organic solvent.

Caution: Plug the column securely when not in use to prevent the column from drying out.