

Size Exclusion Chromatography FAQs

To learn more about
Biozen Size Exclusion
Chromatography go to:

[www.phenomenex.com/
dSEC](http://www.phenomenex.com/dSEC)

What is the optimal salt concentration for good peak shape in size exclusion methods?

Optimal salt concentration can vary depending on the nature of the compound in question. Typically, 150-250mM works best, however you must balance the ionic and hydrophobic nature of the compound. The lower the salt concentration, the more ion-exchange will be observed. At higher salt concentration, more hydrophobic adsorption is observed due to salting of the protein. We typically recommend the customer uses 200mM potassium phosphate and 250mM KCl at pH 6.2 as a starting point to achieve the best results. Potassium is the preferred counter ion as it generates better peak shape and reduces interactions with any silanols at the surface of the particle.

How do I determine the loading capacity of the Biozen SEC column?

For size exclusion, there are two considerations - sample volume and sample concentration. As a general rule, load no more than 5% of the column volume. Theoretically, a 300 x 4.6mm column, with a column volume of ~ 5mL, would limit injection volume to 200 μ L. In practice, volumes of 10-30 μ L are common. Another important consideration is sample concentration; the higher the concentration of protein, the higher the viscosity of the sample. This difference in viscosity can lead to peak shape distortion. The distortion can occur either through exclusion effects or a solvent front referred to as "viscous fingering." A good starting concentration is 1 mg/mL, though optimal concentrations must be determined experimentally.

What is the maximum concentration of salt I can run on a Biozen SEC column?

We recommend 1 M as the maximum salt concentration that should be used in the mobile phase with Biozen SEC columns. Theoretically, a higher salt concentration should be acceptable and would not be incompatible with the columns. However, as salt molarity increases, hydrophobic interactions increase, protein solubility decreases and column backpressure increases.

What is the lifetime of a Biozen SEC column?

SEC column lifetime will vary depending on the method running conditions and sample, as well as column failure definition. The most common failure mode for SEC columns is a column "void," wherein the column bed is compressed and the column can no longer be revived and must be replaced. That said, under typical SEC conditions, and provided both sample and mobile phase have been filtered with a 0.45 or 0.2 μ m filtration device, one should expect ~300 injections.

Can you help me to transfer my current method and/or develop a new method on Biozen SEC columns?

Yes! You may live chat with our technical team at:
www.phenomenex.com/technicalsupport

What are the Biozen SEC shipping solvents?

Unless otherwise noted on column tag are shipped in 0.1 M Phosphate Buffer pH 6.8 with 0.025 % NaN₃.



How do I store my Biozen SEC Column?

It is very important to make sure that your column is clean before storage. This includes removal of buffer, salts, sample, and ion-pairing agents. The recommended storage conditions are:

- Overnight storage: run mobile phase at low flow rate, e.g. 0.2 mL/min for 7.8 mm ID.
- Prolonged storage: column can be stored with 0.1 M NaH_2PO_4 / 0.025 % NaN_3 in water or 20 % methanol in water.
- Flush column with approximately 5 column volumes of 100 % water when switching between mobile phase and storage solvent.

What do you recommend for column installation?

Mobile phase starting conditions check list:

1. Ensure that HPLC grade mobile phase is well mixed, filtered, and degassed prior to use.
2. With the high salt conditions used for SEC methods, precipitation may occur if high organic concentration solvents are left in the LC system. As such, ensure that column shipping solvent, remaining solvent in LC, and mobile phase solvents are miscible.

What are the Biozen SEC material characteristics?

Phases	Description	Pore Size (Å)	Surface Area (m ² /g)	Carbon Load (%)	pH stability	Shipping Solvent	Max Pressure (psi/bar)	Temp (°C)	Mode of Analysis
Biozen 1.8 µm dSEC-2	Inert, high strength porous particle for the separation and quantitation of monoclonal antibody aggregate and fragments	200	–	–	2.5-7.5	0.1 M Sodium Phosphate, pH 6.8 w/ 0.025 % NaN_3	8000/570	50	SEC/GFC
Biozen 3 µm dSEC-2	Inert, high strength porous particle for the separation and quantitation of monoclonal antibody aggregate and fragments	200	–	–	2.5-7.5	0.1 M Sodium Phosphate, pH 6.8 w/ 0.025 % NaN_3	4000/285	50	SEC/GFC

3. Ensure that mobile phase has been filtered using a 0.45 or 0.2 µm filtration device. This is especially critical for sub-2 µm SEC columns which will be more prone to particulate clogging. Prior to column installation, set the maximum pump flow gradient (i.e. mL/ min per minute) to an acceptable level for SEC columns. The default setting can be as high as 100 mL/min min per and this can be particularly deleterious to SEC columns. Consult your HPLC instrument vendor for further guidance on recommended pump settings.

To avoid unnecessary shock to the packed column bed upon installation, set flow rate reasonably low (e.g. 0.1 mL/min for 4.6 mm ID), ensuring the arrow is in the direction of flow. Gradually increase to method flow rate over 5-10 minutes; 90 µL/min for 2.1 mm ID, 0.35 mL/min for 4.6 mm ID, or 1.0 mL/min for 7.8 mm ID.

To learn more about Biozen

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www.phenomenex.com/dSEC

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