biozen by Phenomenex

Size Exclusion Chromatography FAQs

To learn more about Biozen Size Exclusion Chromatography go to:

www.phenomenex.com/dSEC

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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, 150-250 mM 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 compound. We typically recommend the customer uses 200 mM potassium phosphate and 250 mM KCl at pH 6.2 as a starting point to achieve the best results.

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 4.6 mm column, with a column volume of ~ 5 mL, would limit injection volume to 200 µL. In practice, volumes of 10-30 µL 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", where in 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 µm 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 % NaN_3.

Have questions or want more details? We would love to help!
*Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*

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.

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.

Can organic solvents be added to the mobile phase in Size Exclusion Chromatography?

Proteins that are either moderately hydrophobic themselves (membrane proteins) or are Antibody-Drug Conjugates (ADCs) may call for a small amount of organic solvent within the mobile-phase. Things to remember when adding any organic solvent to an SEC method:

- Keep the water-miscible organic solvent within 5 - 20% of the overall mobile-phase
- Be careful of precipitating the phosphate buffering salts or additional salt

Attenuate these concentrations if needed

How does mobile phase effect Size Exclusion Chromatography?

The most common application for SEC is aggregate analysis. It is important not to cause any additional aggregation of a protein by the mobile phase that is used for the analysis. With that in mind conditions of the method are kept close to physiological conditions using a buffered solution at around pH 7 with a moderate amount of salt. A good starting point for mobile phase is a 100 mM Phosphate Buffer, pH 6.8. The concentration of the buffer and the amount of salt added can be modified during method development as they can affect the proteins behavior towards the columns stationary phase.

Phosphate within the concentration range 50 - 200 mM and pH range 6.2 - 7.2

Higher phosphate promotes folding, but also risks salting the protein out of solution and encouraging unwanted hydrophobic retentive interactions with SEC phases.

The high hydrophilicity of the dSEC-2 phase mitigates this behavior at high phosphate concentration, but nothing is invincible.

Additional salt within the concentration range 100 - 300 mM prevents electrostatic interactions between the polar exterior of the folded protein and any residual silanols along the silica-based stationary-phase.

Monitor peak tailing to avoid the use of either too little salt -results in electrostatic retention, too much salt -results in salt out the protein and induce hydrophobic retention.

What are the Biozen SEC material characteristics?

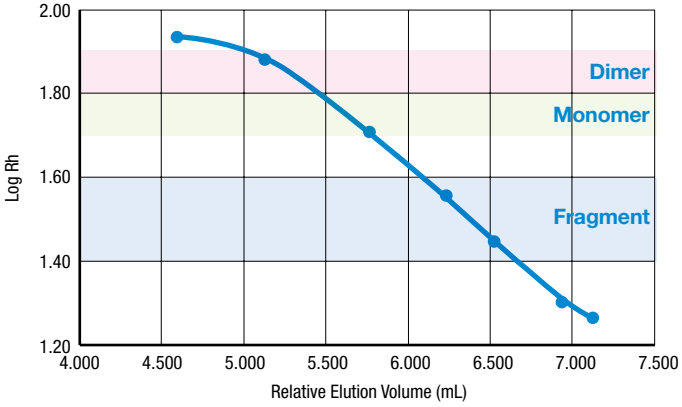
Phases	Description	Pore Size (Å)	Surface Area (m^2/g)	Carbon Load (%)	pH stability	Shipping Solvent	Max Pressure (psi/bar)	Temp (°C)	Mode of Analysis
Biozen 1.8 µm dSEC-2	Inert, high strength porous particle for the separation and quantitation of monoclonal antibody aggregate and fragments	200	–	–	2.5-7.5	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

How do I perform molecular weight calibration for SEC?

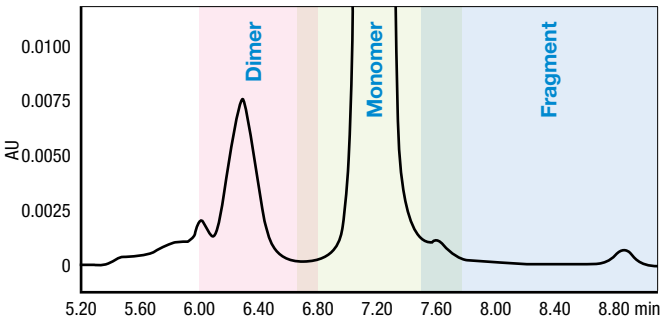
In place of molecular weight, lets instead use the hydrodynamic radius of a biologic compound. Proteins will fold in different ways depending on the conditions they are put in. Due to this the hydrodynamic volume can fluctuate as well thus is a more accurate way to measure proteins than molecular weight.

To calculate the hydrodynamic volume of a protein, first calculate the relative elution volume by subtracting the exclusion void volume, then the logarithm of that will be proportional to the hydrodynamic radius. Example of this calculation can be seen below. Important to note that each SEC column has a range within which this proportional relationship is linear.

Calibration Curve, Protein Standards Elution Volumne (ml) x rh



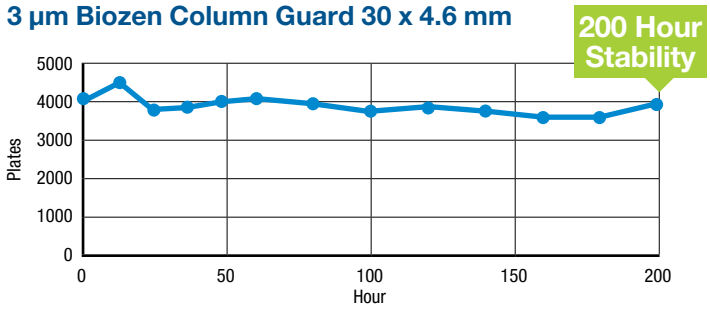
Analyte	Rh (Å)	Log Rh
Dimer	70-80	1.8-1.9
Ig G1 Monomer	50-60	1.7-1.8
Fragment	25-45	1.4-1.6



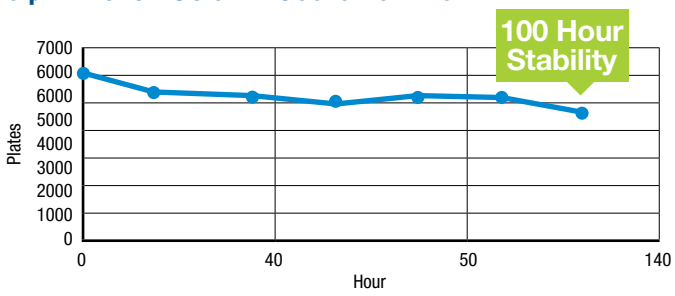
How do Biozen guard columns and cartridges extend column lifetime?

To challenge the bed stability of the dSEC-2 guard column we have tested efficiency over a prolonged period of time continuously running mobile phase shown in the figure below. Sequential injections showed no loss of efficiency for over 100 hours when testing both the 4.6mm and 7.8mm ID. In addition column clogging was tested using a challenging mix of proteins and showed less than a 25% loss of efficiency after 100 hours of use.

3 µm Biozen Column Guard 30 x 4.6 mm



3 µm Biozen Column Guard 40 x 7.8 mm



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