bioZen™
the bio series

bioZen Frequently Asked Questions

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Question	Answer
What is the advantage of running bioZen™ columns at elevated temperatures?	Proteins tend to diffuse very slowly due to their large size, which can make peaks appear broad, even on well optimized columns. By increasing the temperature, the diffusion coefficient of the solvents in the mobile phase are reduced, allowing for faster mass transfer of the protein analytes, resulting in better peak shapes. A further advantage of using high temperature is that selectivity can be manipulated by altering temperature, allowing for better separations in some cases.
What is the maximum molecular weight of protein I can analyze with a bioZen Intact column?	We have successfully achieved chromatographic separation of proteins with molecular weights of 450K on bioZen Intact C4 and XB-C8 columns. It may be possible to run proteins with higher molecular weight, but this would need to be determined experimentally.
Can I switch from non-denaturing to denaturing conditions in SEC and back again?	Commonly used denaturants in SEC included guanidine HCl, urea, and SDS. All of these compounds can be removed from an aqueous size exclusion column, however traces may remain in the column and/or HPLC system. Therefore, it is important to flush thoroughly, and then test the performance of the system with a known protein before continuing with non-denaturing conditions.
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 protein. We typically recommend the customer uses 50 mM potassium phosphate and 250 mM 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.
Can fluorescence detection be used for detection of antibody fractions?	Yes, the Fab fragments fluoresce due to the exposed tryptophan residues. Excitation at 280 nm, with emission at 360 nm is typical for this method. While UV absorbance at 280 nm is possible, fluorescence detection offers greater sensitivity.
Should I use TFA or Formic Acid for my peptide separation on a bioZen Peptide column?	TFA is generally the preferred mobile phase buffer additive for reversed phase separation of peptides and protein. While some researchers prefer to use formic acid buffer where MS detection is used, the preference for TFA in the mobile phase relates to the fact that TFA is a weak ion-pairing buffer.
How should a column be cleaned if it is typically used to analyze protein samples?	A fairly extensive column cleaning procedure may be necessary to completely remove protein contaminants because of the complex nature of protein samples. At each cleaning step, one third to one half of the normal mobile phase flow is recommended to prevent over pressurizing the column. If strong ionic interactions between proteins and the stationary phase are suspected then start cleaning with a denaturant such as 6 M guanidine hydrochloride or 10% DMSO. If the protein is relatively hydrophobic, start by flushing out buffer with 95-100% water, then clean out the hydrophobic proteins with a gradient from 95% water/5% acetonitrile up to 5% water/95% acetonitrile over 3-5 column volumes. If pressure is abnormally high and column frit contamination is suspected, the column can then be reverse-flushed. After using guanidine, the column should be washed with 40-50 column volumes of water or buffer to adequately flush the column. However, there is no guarantee that the proteinaceous material will be completely removed. For more information, please refer to the bioZen column care notes.
What is the most common UV wavelength to detect proteins?	The most common wavelength used for detection of proteins is 280 nm. Absorption of radiation in the near UV by proteins depends on the Tyr and Trp content at this wavelength and to a very small extent the amount of Phe and disulfide bonds.

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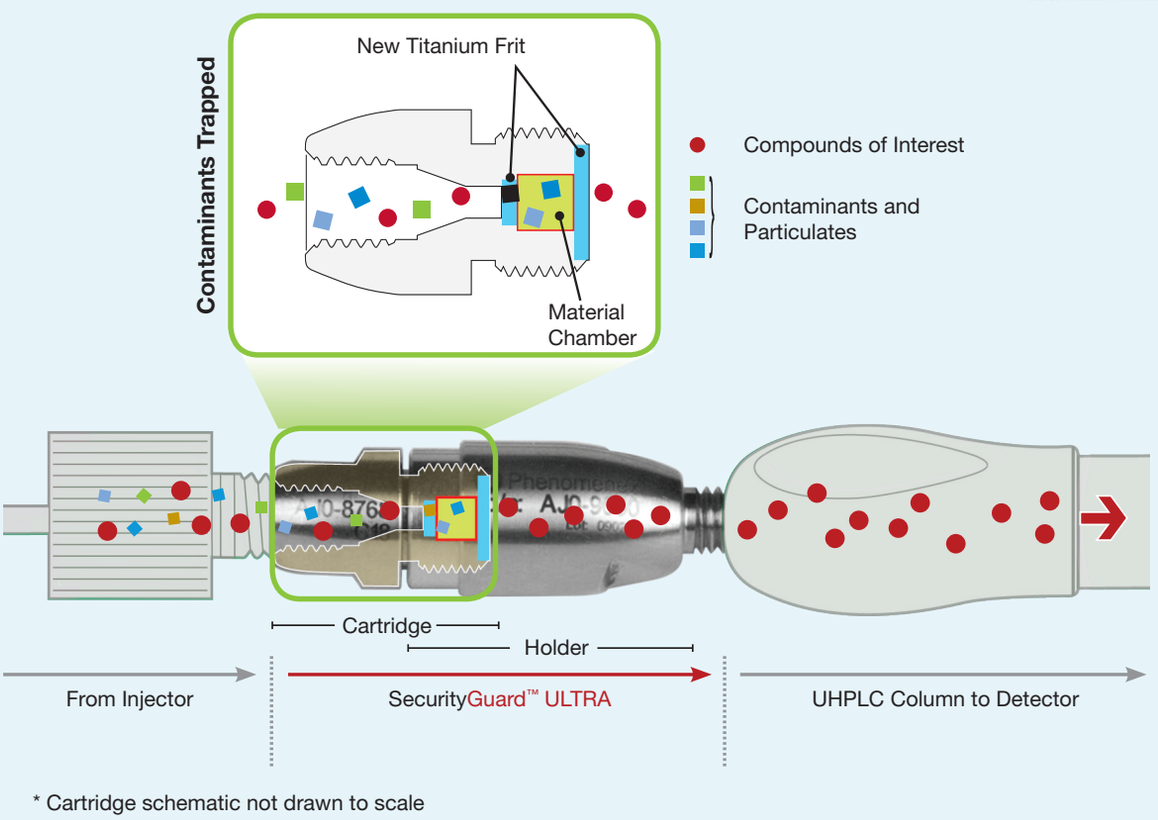
What is the loading capacity of bioZen™ Peptide and Intact columns?	The bioZen Peptide columns have similar loading capacities as reversed phase HPLC/UHPLC columns. A 5-20 µg of digest or peptide mixture on a 4.6 mm ID column will provide good sensitivity especially for LC-MS peptide separations. Up to 50 µg of digest can be loaded without increasing peak width too severely. For 2.1 mm ID columns, loading should be scaled accordingly. For bioZen Intact columns, overloading can drastically affect peak shape due to their lower surface area and must be determined experimentally for optimal results. For 4.6 mm ID's, 5 µg is a good starting point. For 2.1 mm ID's, 1 µg is a recommended starting point. Increasing in load may increase peak tailing and peak width significantly.
What is the lifetime of a bioZen column?	bioZen columns have been rigorously tested at 90 °C for over 1,000 injections without showing a loss in performance.
Can you help me to transfer my current method and/or develop a new method on bioZen columns?	Yes! You may live chat with our technical team at: www.phenomenex.com/technicalsupport
When should I select a bioZen Peptide PS-C18 vs. XB-C18 for peptide mapping?	We recommend that any chromatographer who is analyzing complex peptide mixtures evaluate both columns. The PS-C18 and XB-C18 columns exhibit contrasting selectivity due to the differences in surface chemistry, and chromatographers can utilize these differences to achieve improved resolution between target peptides in different regions of their peptide map.
I am transferring a peptide mapping method to LC-MS and my peak shape is poor. Can I use TFA?	TFA is typically not recommended for any LC-MS methods because it can suppress the ionization of the peptide. However, in some instances, 0.01 % TFA is acceptable to use. Although there may be a decrease in sensitivity, the peptides should still ionize. With that said, if a column has low silanol activity, good peak shapes should be maintained with just 0.1 % formic acid in the mobile phase. Alternatively, 10 mM ammonium formate, pH 3.2 is appropriate as well, and may be more robust chromatographically.
What are some mobile phase considerations for Size Exclusion Chromatography/GFC?	The most common application for SEC is aggregate analysis, which requires "native" conditions (i.e. physiological conditions). These conditions require 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. However, there are some instances where it is appropriate to optimize the mobile phase to improve chromatography. If you suspect that the protein is hydrophobic, a small amount of organic solvent (e.g. methanol, acetonitrile, or isopropanol) can be added generally anywhere between 5-10 %. If you suspect that the protein may have strong ionic characteristics (e.g. basic protein or protein with a high isoelectric point), the ionic strength of the eluent can be increased to improve chromatography. A 2x phosphate buffered saline (i.e. 50 mM phosphate, 300 mM sodium chloride, pH 6.8) is recommended to prevent secondary interactions. Note that increasing the salt will also increase hydrophobic interactions. Further, varying the salt may improve the separation of one critical pair, but result in a loss of resolution of another.
Can sodium azide be used to remove microbial growth?	Although sodium azide can be added to a solution to prevent microbial growth it is not effective at removing growth that has already occurred. In a system that is contaminated with microbial growth it is more effective to flush with 50:50 acetonitrile/water to remove the growth from the system. Note that the column should be flushed with water to remove any buffer salts (e.g. phosphate) before washing with high organic. It may also be necessary to clean or replace guard columns and in-line filters as these are likely to be blocked.

Question	Answer
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.
In GFC, what is a chaotropic agent and how can it be used to determine the molecular weight of a protein?	A chaotropic agent is a compound which disrupts hydrogen bonding in aqueous solution, leading to increased entropy (i.e. chaos). Generally, this reduces the hydrophobic effects that are essential for three dimensional structures of macromolecules such as proteins and nucleic acids. Commonly used chaotropic agents include guanidine or urea, which cause denaturing or unfolding of macromolecules. When used in GFC, chaotropic agents can be used in the mobile phase to determine molecular weight of denatured proteins.
How do you prevent secondary interactions between proteins and stationary phase in GFC?	If secondary interactions are suspected, the mobile phase can be modified to minimize secondary interactions. While some buffer salt (20-100 mM) can help maintain pH in the system and column, there are other additives that can be used to modify secondary interactions. For example, varying NaCl concentration between 0-0.3 M can be used to modulate any ionic interactions between the protein and the stationary phase. Although higher salt results in less ionic interactions between protein and stationary phase, it may also increase hydrophobic interactions. To reduce hydrophobic interactions, salt can be reduced and small amounts of organic (e.g. acetonitrile or isopropanol) can be added to the mobile phase. Alternatively, adjusting the mobile phase pH to either affect silanol interactions (e.g. running under acidic conditions) or affect the net charge of a protein based on protein isoelectric point (pI) can reduce these interactions.
Can I prepare my own low molecular weight protein standards?	Yes. Many low molecular weight protein and peptide standards can be dissolved in 0.1 % TFA/water for analysis on the bioZen SEC columns. Heating is not usually recommended, as it may denature the protein(s) of interest.

Biocompatible Column/System Protection

The easiest way to extend column performance and minimize costly system and detector upkeep is to prevent contaminants and particulates from getting into and past your LC column with a guard system. SecurityGuard guard cartridge systems provide this protection and truly make it easy to acquire this benefit on both HPLC and UHPLC systems.

SecurityGuard ULTRA Guard Cartridge System with Titanium



* Cartridge schematic not drawn to scale

strata X Polymeric SPE

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Without the need for dry-down and reconstitution, Strata-X Microelution SPE 96-well plates provide a consistent sample preparation results with two big benefits: Better absolute recovery and greater time savings.

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Product Ordering Information

bioZen™ Products - Powered by Biocompatible Hardware

bioZen Columns (mm)						Biocompatible Guard Cartridges		
	100 x 2.1	150 x 2.1				for 2.1 mm	for 4.6 mm	Holder
bioZen 2.6 µm Glycan	00D-4773-AN	00F-4773-AN				AJ0-9800	–	AJ0-9000
	50 x 2.1	150 x 2.1				for 2.1 mm	for 4.6 mm	Holder
bioZen 1.6 µm Peptide PS-C18	00B-4770-AN	00F-4770-AN				AJ0-9803	–	AJ0-9000
	50 x 4.6	150 x 4.6				for 2.1 mm	for 4.6 mm	Holder
bioZen 3 µm Peptide PS-C18	00B-4771-E0	00F-4771-E0				–	AJ0-7606	KJ0-4282
	50 x 2.1	150 x 2.1				for 2.1 mm	for 4.6 mm	Holder
bioZen 1.7 µm Peptide XB-C18	00B-4774-AN	00F-4774-AN				AJ0-9806	–	AJ0-9000
	50 x 2.1	150 x 2.1	250 x 2.1	50 x 4.6	150 x 4.6	for 2.1 mm	for 4.6 mm	Holder
bioZen 2.6 µm Peptide XB-C18	00B-4768-AN	00F-4768-AN	00G-4768-AN	00B-4768-E0	00F-4768-E0	AJ0-9806	AJ0-9808	AJ0-9000
	50 x 2.1	150 x 2.1	50 x 4.6	150 x 4.6		for 2.1 mm	for 4.6 mm	Holder
bioZen 3.6 µm Intact C4	00B-4767-AN	00F-4767-AN	00B-4767-E0	00F-4767-E0		AJ0-9809	AJ0-9811	AJ0-9000
bioZen 3.6 µm Intact XB-C8	00B-4766-AN	00F-4766-AN	00B-4766-E0	00F-4766-E0		AJ0-9812	AJ0-9814	AJ0-9000
	150 x 4.6	300 x 4.6				for 2.1 mm	for 4.6 mm	Holder
bioZen 1.8 µm SEC-2	00F-4769-E0	00H-4769-E0				–	AJ0-9850	AJ0-9000
bioZen 1.8 µm SEC-3	00F-4772-E0	00H-4772-E0				–	AJ0-9851	AJ0-9000

guarantee

If bioZen columns in this brochure do not provide at least equivalent separations to a competing column of the same phase, particle size, and dimensions, return the Phenomenex column with comparative data within 45 days for a FULL REFUND.

Ensure Protein Recovery with Biocompatible Accessories!

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