

Tips from our Protein Separation ZenMasters

Loading Capacity for SEC and Reversed Phase HPLC/UHPLC



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How do I determine the loading capacity of a 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 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 loading capacity of bioZen™ Intact and Peptide columns?

The bioZen Peptide columns have similar loading capacities as reversed phase HPLC/UHPLC columns. A 5-20 μ g 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 μ g 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, loading can drastically effect peak shape due to their lower surface area and must be determined experimentally for optimal results. For 4.6 mm ID's, 5 μ g is a good starting point. For 2.1 mm ID's, 1 μ g is a recommended starting point. Increasing in load may increase peak tailing and peak width significantly.

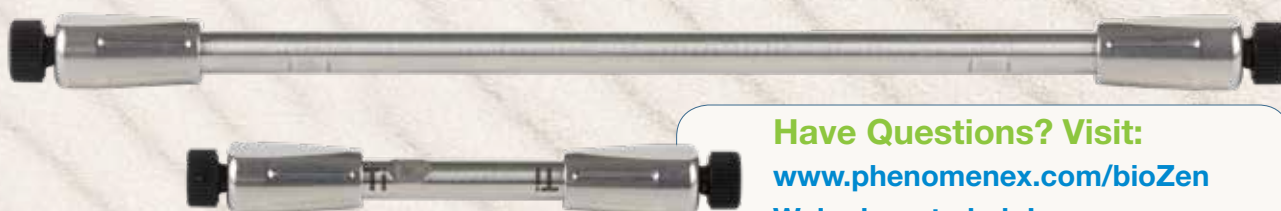


Product Ordering Information

bioZen™ Products - Powered by Biocompatible Hardware

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

*AJO-7606 requires guard holder KJO-4282. All other guard cartridges require guard holder AJO-9000.



Have Questions? Visit:
www.phenomenex.com/bioZen
 We're here to help!

guarantee

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

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