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bioZen™
the bio series

bioZen HPLC/UHPLC Columns Tips for Care and Use

Thank you for purchasing bioZen columns. Below are recommended instructions for the care and use of your bioZen analytical column.

General Information

Each bioZen column manufactured by Phenomenex is individually prepared and tested. Every column is supplied with a Certificate of Quality Assurance (CQA) which indicates testing conditions, operating parameters, and column details. The column details, including specifications and performance test results should be entered into your information management system for easy tracking and reference. Electronic copies of your column's quality documentation can also be acquired at: www.phenomenex.com/mysupport.

Inspection

Upon receipt of column, please verify that the column you received is the one you ordered (i.e. dimension, particle size, media). Additionally, please check the column for any physical damage potentially caused during shipment. Please test the column immediately to verify performance and record the result of your test in your column information management system.

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 2.6 µm Glycan	Provides optimal combination of high efficiency and selectivity for released glycans, suitable for HPLC and UHPLC.	100	200	–	2-7.5	Acetonitrile /0.1 M Ammonium Formate, pH 3.2 (90:10)	8,700/600	60	HILIC
bioZen 1.6 µm Peptide PS-C18	Excellent retention by combined positively charged surface ligand and C18 ligand, contains a positively charged weak base that repels basic ions, suitable for use with UHPLC.	100	260	9	1.5-8.5 ***	Acetonitrile/Water (65:35 v/v)	15,000/1030	90*	RP
bioZen 3 µm Peptide PS-C18	Excellent retention by combined positively charged surface ligand and C18 ligand, contains a positively charged weak base that repels basic ions, suitable for use with HPLC.						5,000/340	90*	RP
bioZen 1.7 µm Peptide XB-C18	Overall retention of both acidic and basic peptides through C18 stationary phase with di-isobutyl side chains, suitable for use with UHPLC.	100	200	10	1.5-9 **	Acetonitrile/Water (65:35 v/v)	15,000/1050	90*	RP
bioZen 2.6 µm Peptide XB-C18	Overall retention of both acidic and basic peptides through C18 stationary phase with di-isobutyl side chains, suitable for use with HPLC and UHPLC.						8,700/600		RP
bioZen 3.6 µm Intact C4	Large pore core-shell particle for fast intact biologic entry. C4 stationary phase provides highly sought after low hydrophobic retention, especially important for highly retentive biologics.	200	20	–			8,700/600		RP
bioZen 3.6 µm Intact XB-C8	Large pore core-shell particle for fast intact biologic entry. C8 provides highly useful moderate hydrophobic selectivity.						8,700/600		RP
bioZen 1.8 µm SEC-2	Extremely inert, high density fully porous particle with high efficiency and low molecular weight (LMW) separation range of 1-450 kDa.	150	–	–	1.5-8.5	0.1 M Phosphate Buffer, pH 6.8 w/ 0.025 % NaN_3	7000/480	50	SEC/GFC
bioZen 1.8 µm SEC-3	Extremely inert, high density fully porous particle with high efficiency and high molecular weight (HMW) separation range of 10-700 kDa.	300	–	–			7000/480	50	SEC/GFC
bioZen 6 um WCX	Monodispersed, non-porous PS-DVB particle with a hydrophilic graft and linear carboxylate polymer chain for the separation of acidic/basic variants for proteins.	–	–	–	2-12	20 mM Sod. Phosphate + 150 mM NaCl 4 mM NaN_3, pH 6.5	6000	60	IEX

* Temperature limits are dependent on method running parameters. The temperature limit of the phase at high pH, for example, would be approximately at 60 ˚C.
** pH range is 1.5 - 9 under gradient conditions. pH range is 1.5 -10 under isocratic conditions.
*** pH range is 1.5 - 8.5 under gradient conditions. pH range is 1.5 -10 under isocratic conditions.

Mobile Phase Compatibility

When using bioZen™ columns (HPLC and UHPLC), use only HPLC grade solvents. Do not use immiscible solvents and buffers.

Additionally, the use of solvent filtration is highly recommended to remove trace impurities from your mobile phase of choice.

Column Installation

System check:

Initial setup of your LC system is very important to ensure column performance:

Ensure the system is ready:

- Seals, liners, injector clean
- Primed lines (checking to make sure there are no bubbles)
- Steady baseline
- Consistent system pressure

Mobile Phase/Solvent Check:

- Check that all solvents are miscible
- Check needle wash and prime solvent bottles/vials are filled with an appropriate solvent and at a good level
- Mobile phase is well mixed, filtered, degassed, freshly prepared if possible

Typical/Maximum Flow Rate:

Flow rates are dependent on internal diameter, particle size, and system pressure tolerance

- bioZen WCX:
  - 50 mm up to 0.5 mL/min
  - 100 mm up to 0.4 mL/min
  - 150 mm up to 0.3 mL/min
  - 250 mm up to 0.2 mL/min
- bioZen SEC-2/SEC-3:
  - 100 mm up to 0.6 mL/min
  - 150 mm up to 0.5 mL/min
  - 300 mm up to 0.4 mL/min
- RP sub-2 µm: 0.3-0.8 mL/min (typical)
- RP, HILIC 2 µm and above: 0.5-2.0 mL/min

Column Installation:

- Keeping in mind pressure limits, set flow rate to 0.1 mL/min
- Ramp up the flow rate to 0.2 mL/min. Let flow into a small beaker for 5 minutes
- Collect and check the flow through with a small beaker, then wipe both ends
- Pause flow. Install the other line (to detector)
- Ramp up the flow rate to method flow

Conditioning & Priming:

- Condition the column for 10-20 column volumes
- Monitor the pressure: a steady pressure should indicate a constant flow while pressure fluctuation will indicate air in the system
- Wide fluctuations in pressure may shock and damage the column
- Monitor the pressure as well as signal from the detector as well as the system. When both are steady, the column is ready for use
- For all gradient methods (RP, HILIC, and IEX) perform at least one blank before analysis

Testing Column Performance

Standards can be used to check the condition of the column. Here are some conditions.

Phases	Part Number	CQA Conditions
bioZen 2.6 µm Glycan	AL0-8317	Diluent: Acetonitrile/Water (85:15) Injection Volume: 1 µL Mobile Phase: Acetonitrile/100 mM Ammonium formate, pH 3.2 (90:10) Flow Rate: 0.5 mL/min UV: 254 nm
bioZen 1.6 µm Peptide PS-C18	AL0-3045	Diluent: Acetonitrile/Water (75:25) Injection Volume: 1 µL
bioZen 3 µm Peptide PS-C18		Mobile Phase: Acetonitrile/Water (65:35) Flow Rate: 0.75 mL/min * UV: 254 nm
bioZen 1.7 µm Peptide XB-C18	AL0-3045	Diluent: Acetonitrile/Water (75:25) Injection Volume: 1 µL
bioZen 2.6 µm Peptide XB-C18		Mobile Phase: Acetonitrile/Water (65:35) Flow Rate: 0.75 mL/min * UV: 254 nm
bioZen 3.6 µm Intact C4	AL0-8931	Diluent: Acetonitrile/Water (50:50) Injection Volume: 0.1 µL
bioZen 3.6 µm Intact XB-C8		Mobile Phase: Acetonitrile/Water (55:45) Flow Rate: 0.25 mL/min UV: 254 nm
bioZen 1.8 µm SEC-2	AL0-9253	Diluent: 100 mM Sodium Phosphate, pH 6.8 Injection Volume: 0.7 µL
bioZen 1.8 µm SEC-3		Mobile Phase: 100 mM Sodium Phosphate, pH 6.8 Flow Rate: 0.35 mL/min UV: 280 nm

* Double check your CQA conditions as flow rate will change with the particle size.

Column Cleaning

Reversed Phase for Intact Analysis:

General Cleaning Procedure:

1. 50:50 Acetonitrile/Water for at least 20 column volumes or when normal pressure is observed.
2. Clogged frits can be removed with reverse flushing (flushing the column with the column reversed).
3. Always inject a blank injection prior to injection to check column. It is also a good idea to inject a blank after.

Alternative Cleaning Procedure:

1. For example, if the last injection ended with Buffer/ Acetonitrile (75:25), its more appropriate to start with 95:5 Water/ Acetonitrile and then move step by step as needed to increase organic content (e.g. 75:25 Water/ Acetonitrile, 50:50 Water/ Acetonitrile, 5:95 Water/Acetonitrile).
2. For hydrophobic or oily materials, try flushing with IPA. When using IPA, ensure use of a low flow to prevent higher backpressures due to higher solvent viscosity.

Reversed Phase Peptide Analysis:

General Cleaning Procedure:

1. 50:50 Acetonitrile/Water for at least 20 column volumes or when normal pressure is observed.
2. Clogged frits can be removed with reverse flushing (flushing the column with the column reversed).
3. Always inject a blank injection prior to injection to check column. Though not necessary, it is also a good idea to inject a blank after the cleaning as well.

Alternative Cleaning Procedure:

1. Hydrophobicity is typically not an issue with peptide analysis but you can use IPA to remove the larger chains and other hydrophobic components.

HILIC:

General Cleaning Procedure:

1. 50:50 Acetonitrile/Water for at least 20 column volumes or when normal pressure is observed.

SEC/GFC:

General Cleaning Procedure:

1. Start with 10 column volumes of 0.1 M NaH_2PO_4 buffer, pH 3.0. Follow with at least 10 column volumes of 100 % HPLC grade water.

   Reverse flushing is acceptable, but please reduce flow rate to low flow (e.g. 1 mL/min method flow to 0.5 mL/min). Hydrophobic Analytes: Set gradient clean from 100 % water to 100 % acetonitrile for 30 mins, at low flow.

   Then gradient clean from 100 % acetonitrile to 100 % water for 30 mins, at low flow.
2. Flush with 100 % water overnight for 12 hours.

Removal of Strongly Adsorbed Proteins:

1. Wash with 30 mL of 0.5 % SDS, 6 M Guanidine thiocyanate or 10 % DMSO for 30 mins at a reduced flow rate.
2. Immediately flush with water overnight at low flow.

Column Regeneration:

1. After exposure to denaturants (SDS, Guanidine thiosulfate, urea), flush with water overnight at low flow.
2. Test column with Aqueous SEC manufacturing standard after denaturants to verify column performance. Then trial inject your standard to further confirm.

Weak Cation Exchange:

General Cleaning Procedure:

Wash the column with 10-20 column volumes of a high salt eluent (e.g. buffer with 1 M NaCl) to remove any strongly retaining compounds. Ensure that backpressure is within limitations of the column; lower flow-rate appropriately.

Alternative Cleaning Procedure:

For proteins that still retain after high salt eluent cleaning procedure, wash the column with 5-10 column volumes of 0.1 N HCl. Equilibrate in 20 mM MES, pH 6.5 or starting mobile phase conditions.

Wash the column with 5-10 column volumes of 20 mM NaOH. Equilibrate in 20 mM MES, pH 6.5 or starting mobile phase conditions.

For hydrophobically retained compounds, wash the column with 5-10 column volumes of 50% Acetonitrile. Equilibrate in 20 mM MES, pH 6.5 or starting mobile phase conditions.

Avoid protic solvents (i.e. alcohols like methanol), cationic detergents, extremes in pH and temperature.

Column Storage

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:

Phases	Storage Conditions	Comments
bioZen™ 2.6 µm Glycan	Acetonitrile/Water (80:20 v/v)	Methanol is not recommended to use for storage.
bioZen 1.6 µm Peptide PS-C18	Acetonitrile/Water (50:50 v/v)	Methanol can be used instead of Acetonitrile but equilibration will take longer.
bioZen 3 µm Peptide PS-C18		
bioZen 1.7 µm Peptide XB-C18		
bioZen 2.6 µm Peptide XB-C18		
bioZen 3.6 µm Intact C4	Acetonitrile/Water (20:80 v/v)	Methanol can be used to store instead of Acetonitrile but equilibration will take longer.
bioZen 3.6 µm Intact XB-C8	Acetonitrile/Water (20:80 v/v)	
bioZen 1.8 µm SEC-2	HPLC grade Water	For prolonged storage, 0.1 M NaH_2PO_4 / 0.025 % NaN_3 in water or 20% Methanol in water can be used.
bioZen 1.8 µm SEC-3	HPLC grade Water	
bioZen 6 µm WCX	20 mM MES buffer, pH 6.5	For long term storage use 20 mM MES buffer, pH 6.5+ 0.05% Sodium Azide

Tips for Extending Column Lifetime

Proper sample preparation can help maintain the system and column lifetime.

- Filter samples, when appropriate (i.e. Phenex™ Syringe Filters)
- Use appropriate guard column and guard cartridge systems
- Work at appropriate sample levels, do not overload the column
- Work in the appropriate separation mode for the column, check column characteristics prior to analysis for allowable parameters
- Store your column in appropriate solvents
- For SEC typical load is 0.1% to 0.5% of the column volume

Amounts of Sample That Can Be Separated

Column Type	ID (mm)	Approx. Dead Volume (mL)*	Typical Flow Rate (mL)	Typical and (Max.) Injection Masses (mg)	Typical and (Max.) Injection Volumes (µL)**
Analytical	4.6	1.5	0.5 - 2.0	0.1 (2.5)	10 (200)

* The column Dead Volume (Vo) may be estimated from:
Column Dead Volume (mL) = Vo = 0.487 x d^2 x L
Where: L = column length (cm); 15 cm (150 mm) used for calculation.
d = column ID (cm, not mm)

** The maximum allowable Sample Injection Volume (Vi) can be estimated as follows:

$$\text{Maximum Injection Volume} = Vi = \frac{Vr}{2\sqrt{N}}$$

Where: Vr = the retention volume of the first peak (mL)
N = number of theoretical plates per column

Column Warranties

Phenomenex HPLC columns are warranted to meet the stated performance and quality and to be free of defects in material and workmanship. If you are unsatisfied for any reason, please give your Phenomenex Technical Representative a call. We'll do our best to solve the problem to your satisfaction. Should it become necessary to return the column, a Return Authorization Number must be obtained from Phenomenex first.

Disclaimers

New columns should be tested with the manufacturers recommended test mix, and previously used columns should be tested with the same or a suitable test mix for the analysis. Remember to re-equilibrate the system when changing solvents. Never change from one solvent to another which is immiscible, without going through an intermediate solvent which is miscible with both. This will damage the column. Never change to (or from) a buffer/salt solution where the buffer/salt is not soluble in the second solvent. Again, this will damage the column. Never attempt to remove the column end fittings. This will void the warranty.

Column Shock

Handle columns with care. Do not drop or create physical shock. Do not start pump at high flow rates, instead ramp up gradually over a few minutes. Set your pump pressure limit to protect the column in event of blockage. This can create voids which will detrimentally affect the column's performance.

For any additional questions visit:
Phenomenex.com/chat
or submit an inquiry to:
support.phxtechnical@zendesk.com

For more information on bioZen™ HPLC and UHPLC columns, please visit **www.phenomenex.com/biozen**

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