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/technicalsupport.

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 1.7 µm Oligo	The particle is an organo-silica core-shell bonded with a C18 stationary phase.	100	200	11	1-12	Acetonitrile/Water (60:40)	15,000/1050	60	RP
Biozen 2.6 µm Oligo	The particle is an organo-silica core-shell bonded with a C18 stationary phase.						8,700/600		
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		
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		
Biozen 3.6 µm Intact XB-C8	Large pore core-shell particle for fast intact biological entry. C8 provides highly useful moderate hydrophobic selectivity.	200	20	–	1.5-9**	Acetonitrile/Water (65:35 v/v)	8,700/600	90*	RP
Biozen 2.6 µm WidePore C4	Core-shell particle with butyl stationary phase and optimal wide pore size distribution for better resolution of large biologics, including monoclonal antibodies and sub-unit analysis	400	25	–	1.5-9**	Acetonitrile/Water (65:35 v/v)	12500	90*	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	–	–	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 dSEC-2	Inert, high-strength porous particle for the separation and quantitation of monoclonal antibody, biosimilar and biothera peutic aggregate and fragment analysis.	200	–	–	2.5-7.5	0.1 M Sodium Phosphate, pH 6.8 w/ 0.025 % NaN_3	8000/550	50	SEC/GFC
Biozen 3 µm dSEC-2	Inert, high-strength porous particle for the separation and quantitation of monoclonal antibody, biosimilar and biothera-peutic aggregate and fragment analysis.		–	–			4000/275		
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 Sodium Phosphate + 150 mM NaCl 4 mM NaN_3, pH 6.5	6000	60	IEX

* Temperature limits are dependent on method parameters. For example, at the pH upper limit the temperature limit would be approximately 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.

Column Installation

System check:

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

Ensure the system is ready:

- Ensure that seals, liners, and injector are clean
- Confirm primed lines are free of air bubbles
- Establish stabilized baseline
- Establish stabilized system pressure

Mobile Phase/Solvent Check:

- Use only HPLC grade quality or better solvents to maximize column performance
- Ensure that all solvents are miscible
- Ensure mobile phase is well mixed, degassed, and freshly prepared if possible
- For SEC mobile phases that are susceptible to microbial growth, avoid refilling existing mobile phase bottles. Instead utilize new bottles with freshly prepared mobile phase
- For SEC mobile phases, avoid the use of solvent inlet filters as these are often sources of microbial growth
- Filter aqueous buffers with a 0.2 µm filter
- Check that needle wash and prime solvent bottles/vials are filled with appropriate solvent at sufficient volumes

Column Installation:

- Start by connecting the inlet end of the column to the injector outlet
- Set initial flow rate to 0.1 mL/min
- Gradually increase flow rate to 0.2 mL/min. Allow column outlet is achieved to flow into a small beaker for 5 minutes
- Ensure constant flow from column outlet, then wipe both ends
- Pause flow. Install column outlet line to detector
- Increase flow rate to method flow rate and ensure backpressure and baseline have stabilized before moving to next step

Condition/Equilibration:

- Condition the column for 10-20 column volumes of starting mobile phase conditions
- HILIC column chemistries should be conditioned for at least 20-30 column volumes
- Once a stabilized backpressure and signal from the detector are observed the column can be considered equilibrated
- To avoid precipitation of mobile phase buffers, it is recommended that columns be flushed with 5 column volumes of non-buffered starting conditions first (e.g. if starting conditions are 50/50 buffered solution/Acetonitrile it is suggested to run 50/50 Water/Acetonitrile to start)
- Best practices suggest performing at least one blank injection before analysis to ensure the system and mobile phase are free of contaminants

Recommended/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 d-SEC2/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
- Biozen RP: 0.3-0.8 mL/min
- Biozen HILIC: 0.5-2.0 mL/min

Testing Column Performance

Performance reference standards can be used to verify and trend column performance over time.

Phases	Part Number	CQA Conditions
Biozen 1.7/2.6 µm Oligo	AL0-3045	Diluent: Acetonitrile/Water (75:25) Injection Volume: 1 L Mobile Phase: Acetonitrile/Water (65:35) Flow Rate: 0.75 mL/min * UV: 254 nm
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 1.6/3 µm Peptide PS-C18	AL0-3045	Diluent: Acetonitrile/Water (75:25) Injection Volume: 1 µL Mobile Phase: Acetonitrile/Water (65:35) Flow Rate: 0.75 mL/min * UV: 254 nm
Biozen 1.7/2.6 µm Peptide XB-C18	AL0-3045	Diluent: Acetonitrile/Water (75:25) Injection Volume: 1 µL Mobile Phase: Acetonitrile/Water (65:35) Flow Rate: 0.75 mL/min * UV: 254 nm
Biozen 2.6 µm WidePore 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
Biozen 1.8/3 µm dSEC-2	AL0-9253	Diluent: 100 mM Sodium Phosphate, pH 6.8 Injection Volume: 1.40 µL Mobile Phase: 100 mM Sodium Phosphate, pH 6.8 Flow Rate: 0.35 mL/min UV: 280nm
Biozen 6 um WCX	Equine Cytochrome C**	Diluent: 20 mM Sodium Phosphate, 150 mM Sodium Chloride, 4 mM Sodium Azide, pH 6.5 Injection Volume: 5 uL Mobile Phase: 20 mM Sodium Phosphate, 150 mM Sodium Chloride, 4 mM Sodium Azide, pH 6.5 Flow Rate: 1.3 mL/min UV: 280 nm

* Double check your CQA conditions as flow rate will change with the particle size.
** Check standard was sourced from Sigma-Aldrich.

Column Cleaning

Always utilize a cleaning procedure that most closely aligns with the properties of your suspected contaminants and/or analytes of interest.

Reversed Phase for Intact Analysis:

General Cleaning Procedure:

1. Flush columns with 50:50 Acetonitrile/Water for at least 20 column volumes or until normal backpressure is observed.
2. Making a blank injection before and after your cleaning procedure will ensure the system and mobile phase are not contributing to observed chromatographic artifacts.

Alternative Cleaning Procedure:

1. If further cleaning is required, start with conditions at the end of your chromatographic run and increase the amount of organic solvent stepwise. It is recommended that non-buffered solutions be utilized to avoid precipitation at higher concentrations of organic.
2. For increasingly hydrophobic contaminants IPA can be used. When using IPA, ensure a lower operating flow rate is used to prevent elevated backpressure due to higher solvent viscosity.

Reversed Phase Peptide Analysis:

General Cleaning Procedure:

1. Flush columns with 50:50 Acetonitrile/Water for at least 20 column volumes or until normal backpressure is observed.
2. Making a blank injection before and after your cleaning procedure will ensure the system and mobile phase are not contributing to observed chromatographic artifacts.

Alternative Cleaning Procedure:

1. Hydrophobic contaminants are typically not an issue with peptide analysis but IPA can be utilized if suspected. Ensure a lower operating flow rate is used to prevent elevated backpressure due to higher solvent viscosity.

HILIC:

General Cleaning Procedure:

1. Flush columns with 50:50 Acetonitrile/Water for at least 20 column volumes or until normal back pressure is observed.
2. If backpressure does not stabilize or polar contaminants are still suspected, flush column with 5:95 Acetonitrile/Water.

Oligo:

General Cleaning Procedure:

1. Start with conditions at the end of your last chromatographic run and increase the amount of organic solvent stepwise. It is recommended that non-buffered solutions be utilized to avoid precipitation at higher concentrations of organic.
2. For increasingly hydrophobic contaminants IPA can be used. When using IPA, ensure a lower operating flow rate is used to prevent elevated backpressure due to higher solvent viscosity.
3. When highly hydrophobic contaminants are suspected, THF may be utilized. Ensure that your system is compatible and reduce both operating flow rate and temperature.
4. For methods utilizing ion pairing reagents such as alkylamine, the column may need to be re-equilibrated after cleaning. It is recommended to equilibrate the column for 30-40 column volumes. More column volumes may be required when utilizing lower ion-pairing concentrations (<2 mM).

Tips:

If the cleaning procedures above have been attempted but an increase in back pressure is still observed, and/or clogged frits are suspected, you can try reverse flushing the column with the reduced flow rates indicated below:

1. 0.1 mL/min (2.1 mm diameter column).
2. 0.3 mL/min (3.0 mm diameter column).
3. 0.5 mL/min (4.6 mm diameter column).

SEC/GFC:

General Cleaning Procedure:

1. Flush column 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.
2. Reverse flushing may be attempted. Reduce flow rate to half of normal flow rate conditions. If hydrophobic contaminants are suspected, ramp gradient at low flow from 100% Water to 100% Acetonitrile over 30 minutes and then back to 100% water over 30 minutes.

Removal of Strongly Adsorbed Proteins:

1. Wash with 10-20 column volumes of 0.5% SDS, 6 M Guanidine thiocyanate or 10% DMSO at a reduced flow rate.
2. Immediately flush with water overnight at low flow.

Weak Cation Exchange:

General Cleaning Procedure:

1. Wash the column with 10-20 column volumes of a high ionic strength solution (e.g. buffer with 1 M NaCl) to remove any strongly retained compounds. Monitor backpressure and lower flow rate to meet column specifications if needed.

Alternative Cleaning Procedure:

1. For proteinaceous contaminants that still retain after high ionic strength cleaning procedure, wash column with 5-10 column volumes of 0.1 M HCl. Equilibrate in 20 mM MES, pH 6.5 or starting mobile phase conditions.
2. If further cleaning is required, 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.
3. For increasingly hydrophobic 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.
4. Avoid utilzing protic solvents (e.g. alcohols like methanol), cationic detergents, and extreme pH and temperature conditions.

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 reagents. The recommended storage conditions are:

Phases	Storage Conditions	Comments
Biozen 1.7/2.6 µm Oligo	Acetonitrile/Water (50:50 v/v)	Methanol can be used instead of Acetonitrile but equilibration will take longer.
Biozen 2.6 µm Glycan	Acetonitrile/Water (80:20 v/v)	Methanol is not recommended to use for storage.
Biozen 1.6/3 µm Peptide PS-C18	Acetonitrile/Water (50:50 v/v)	Methanol can be used instead of Acetonitrile but equilibration will take longer.
Biozen 1.7/2.6 µm Peptide XB-C18		
Biozen 2.6 µm WidePore 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		
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. Flush column with approximately 5 column volumes of 100% water before switching between mobile phase and storage solvent.
Biozen 1.8 µm SEC-3		
Biozen 1.8/3 µm dSEC-2	Run mobile phase at low flow rate over night	For prolonged storage, 0.1 M NaH2PO4/ 0.025% NaN3 in water or 20% methanol in water can be used. Flush column with approximately 5 column volumes of 100% water before switching between mobile phase and storage solvent.
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 maximize the system performance and column lifetime.

- Filter samples, when appropriate (i.e. Phenex™ Syringe Filters)
- Use appropriate guard column and guard cartridge systems
- Work at appropriate sample loads; do not overload the column
- Work in the appropriate method conditions for the column consult column specifications prior to analysis
- Store your column in appropriate solvents
- For SEC typical load is 0.1 % to 0.5 % of the column volume

Establish Appropriate Loading and Injection Volumes

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**

GU52850423_W