Foresight Pro Column

User Guide

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BIO-RAD

Foresight Pro Chromatography Columns

User Guide

BIO-RAD

Bio-Rad Technical Support Department

The Bio-Rad Technical Support department in the U.S. is open Monday through Friday, 5:00 AM to 5:00 PM, Pacific time.

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For technical assistance outside the U.S. and Canada, contact your local technical support office or click the Contact us link at www.bio-rad.com.

Legal Notices

Revision History

Document	Date	Description of Change
Foresight Pro Columns User Guide Bulletin 3273 Ver B	December 2022	In the Column Construction Materials section, change the EMEA 410/01 entry to animal-free for bed support screens
Foresight Pro Columns User Guide Bulletin 3273 Ver A	May 2022	New document

Table of Contents

Safety and Regulatory Compliance

To avoid personal injury or damage to equipment, you must use the columns in accordance with the following:

- Standard Operating Procedures (SOPs) for using equipment and associated components in your facility
- Safety instructions and product use requirements provided in this document

Important: It is the employer's responsibility to ensure safe operation, and to provide appropriate information and training to users. The instructions provided herein are suggested best working practices when using Foresight Pro columns, and are not meant to take precedence over company SOPs and local regulations.

Regulatory Compliance

Foresight Pro column components that are in contact with fluids meet one or more of the following regulatory requirements:

- FDA 21 CFR 177.2600 — Rubber articles intended for repeated use
- USP <88> Class VI — BIOLOGICAL REACTIVITY TESTS, IN VIVO
- EMEA 410/01 Rev 3.1 July 2011— Note for guidance on minimizing risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products

Safety and Information Labels

Foresight Pro columns are designed to operate safely when used in the manner prescribed by the manufacturer and in this user guide. Specific labels used in this guide are described in Table 1.

Important: You must comply with all SOPs and safety regulations specified for unpacking, handling, and using the product.

Table 1. Important and standard note identifiers

Icon	Meaning
	This label indicates where you should exercise particular care.
	This label indicates an item requiring special attention.

General Handling Precautions

Adhere to the following guidelines when working with Foresight Pro columns.

Important: The safety pressure limit for the column hardware is 5 bar. See Table 2 in Product Usage Requirements on page 12 for operating pressure.

- Follow your SOPs for lifting heavy objects.
- Use common laboratory safety equipment to prevent exposure to biohazardous reagents reaching eyes or mouth. For example, use a face shield when operating the equipment.
- Always wear laboratory gloves, coats, head coverings where applicable, and safety glasses with side shields or goggles.
- Keep your hands away from your mouth, nose, and eyes.
- Completely protect any cut or abrasion before working with potentially infectious materials.
- Wash your hands thoroughly with soap and water after working with any potentially infectious material before leaving the laboratory.
- Remove wristwatches and jewelry.
- Store all infectious or potentially infectious material in unbreakable leak-proof containers.
- Before leaving the laboratory, remove protective clothing.
- Do not use a gloved hand to write, answer the telephone, turn on a light switch, or touch anything that other people may touch without gloves.
- Change gloves frequently. Remove gloves immediately when they are visibly contaminated.
- Do not expose materials that cannot be properly decontaminated to potentially infectious material.
- Upon completion of any operation involving biohazardous material, decontaminate the work area with an appropriate disinfectant.

Chapter 1 About Foresight Pro Columns

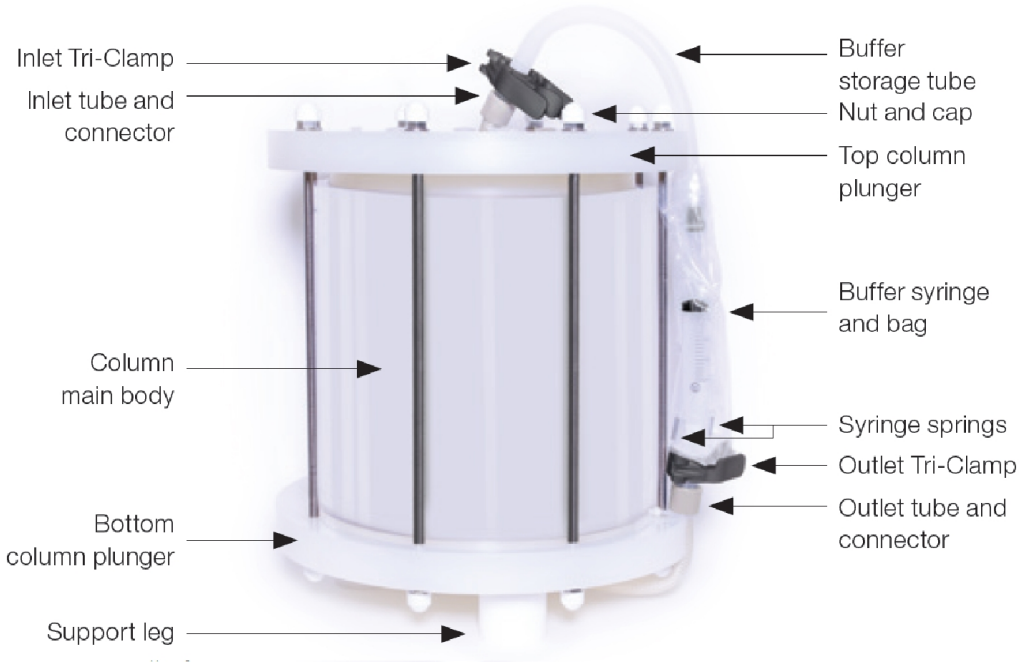
Foresight Pro columns are designed for chromatographic purification of biological molecules in Good Manufacturing Practice (GMP) production processes. This includes, but is not limited to, purification and polishing applications for vaccines, monoclonal antibodies, and recombinant proteins.

The columns are manufactured from polymeric materials that provide the biological and chemical compatibility with biopharmaceutical manufacturing applications. Their sanitary design includes minimum dead spaces and effective cleanability.

The columns are GMP ready and prepacked with resin as the stationary chromatography phase.

Product Design

The following picture illustrates the main components of Foresight Pro columns.



Product Usage Requirements

Table 2 contains requirements for using Foresight Pro columns with your chromatography system.

Important:

- Do not use alcohols over 20% concentration (v/v) on or with Foresight Pro columns.
- To avoid disrupting the packed CHT bed and generating a headspace, operate the column at 3 bar or less.

Table 2. Foresight Pro chromatography columns packed with CHT media

Parameter	Requirement
Operating temperature	2° C to 25° C
Operating pressure	3 bar maximum
Storage temperature	Cold room (1° C to 10° C) ;to room temperature (22 ° C)
Relative humidity	5-80% non-condensing

Column Construction Materials

Foresight Pro columns are designed for a vast range of biopharmaceutical manufacturing applications, and are made of materials that provide the best biological and chemical compatibility.

Table 3 contains non-product contact materials and Table 4 contains product contact materials. For definitions of terms and acronyms, see Appendix C, Glossary of Terms.

Table 3. Non-product contact materials

Element	Material
Top and bottom supporting plates:	Polypropylene
Holding bolt	Polyamide or 304 stainless steel

Table 4. Product contact materials

Component	Material	USP <88> Biological Reactivity Test	CFR 21 177	Animal Origin
Column body (5 cm ID columns)	Glass	N/A	N/A	N/A
Column body (8, 10, 13, 20, 24, 33 cm ID columns)	Acrylic	Class VI	177.1010	Animal-free
Flow distributors	Polypropylene	Class VI	177.1520	Animal-free
Inlet and outlet connectors	Polypropylene	Class VI	177.1520	Animal-free
Tubing connections	Reinforced elastomer	Class VI	177.2600	Animal-free
O-rings	Viton	Class VI	177.2600	Animal-free
Tri-Clamp gasket	EPDM	Class VI	177.2600	Animal-free
Bed support screens	Polyamide	Class VI	177.1500	Animal-free

Note: The polyamide bed support screen is welded to the polypropylene flow distributor without using any extra chemicals.

Column Specifications By Diameter

Foresight Pro columns are available with CHT Type I, CHT Type II, and CHT XT media, with 40 µm particle size. Table 5 contains specifications for all column sizes by inner diameter.

Note: All connectors are Tri-Clamps.

Table 5. Column specifications

Column inner diameter (cm)	Internal cross-section (cm^2)	Bed height (cm)	Bed volume (L)	Overall dimensions (cm)	Total column weight (kg)	Flow path internal diameter (mm)
5	19.6	10	0.2	30 x 15 x 15	1.8	3.18
5	19.6	20	0.4	40 x 15 x 15	2	3.18
8	50	10	0.5	30 x 15 x 15	2.5	3.18
8	50	20	1.0	40 x 15 x 15	3	3.18
10	78	10	0.8	32 x 17 x 17	3.7	6.35
10	78	20	1.5	42 x 17 x 17	4.5	5.35
13	130	10	1.3	32 x 20 x 20	4.7	6.35
13	130	20	2.7	42 x 20 x 20	6	6.35
20	314	10	3.1	37 x 34 x 34	12	9.53
20	314	20	6.3	46 x 34 x 34	16	9.53
24	452	10	4.5	37 x 39 x 39	18	9.53
24	452	20	9	47 x 39 x 39	25	9.53
33	855	10	8.5	37 x 50 x 50	34	9.53
33	855	20	17	47 x 50 x 50	45	9.53

Chapter 2 Handling the Column

This section describes how to remove the column from its packaging. Large columns (24 and 33 cm) are shipped in wooden crates, and column sizes 20 cm and smaller are shipped in boxes. Foam inserts and plastic overwrap are used for safe shipping.

Before you begin, ensure the following:

- You have removed the shipping documents from the box or crate exterior.
- You are properly attired according to safety regulations. For information, see General Handling Precautions on page 9.
- At least one other person is available to assist with physical lifting of large columns.
- You are using appropriate lifting devices, carts, and tools where applicable.

Removing a Column From a Box

Columns smaller than 24 cm are shipped in boxes, surrounded by foam protectors. This section describes how to remove the internal packaging and the column without damage.

Important:

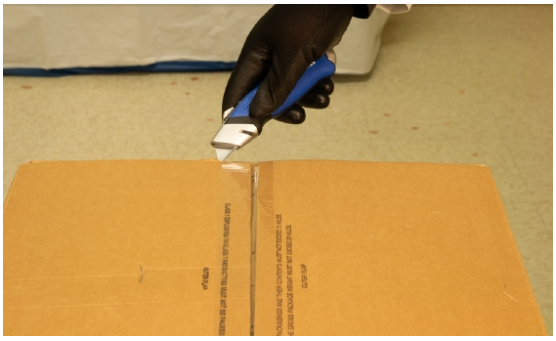
- The column is GMP ready. Follow your SOPs for handling GMP ready products. Two people might be required for lifting.
- To avoid injury and damage, use care when placing the column in a secure area. If you are placing the column on a rolling cart, ensure the column is secure in the center, and in a vertical position.

To open the box and remove the column

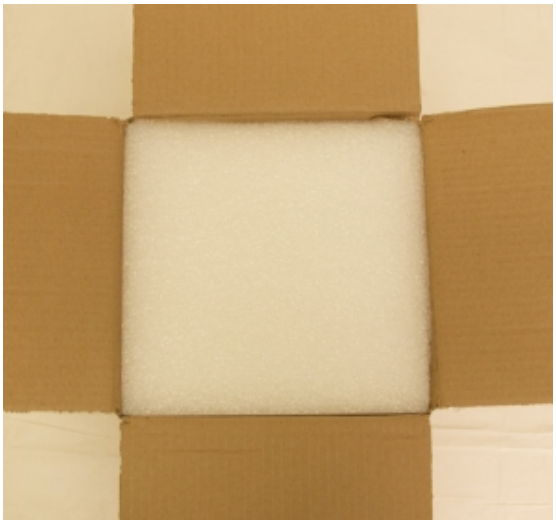
1. Inspect the package exterior through the plastic wrap. If you see any damage, contact Bio-Rad Technical Support at 1-800-424-6723, option 2 or support@bio-rad.com (U.S./Canada Only).

2. Cut and remove the plastic wrap, and then locate and remove the external documents.

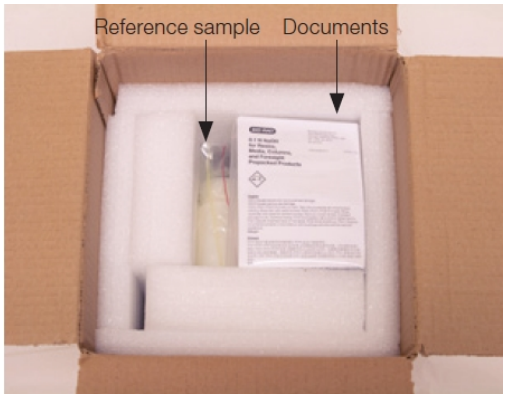
3. Break the seal to open the box.



4. Remove the foam covering above the contents.



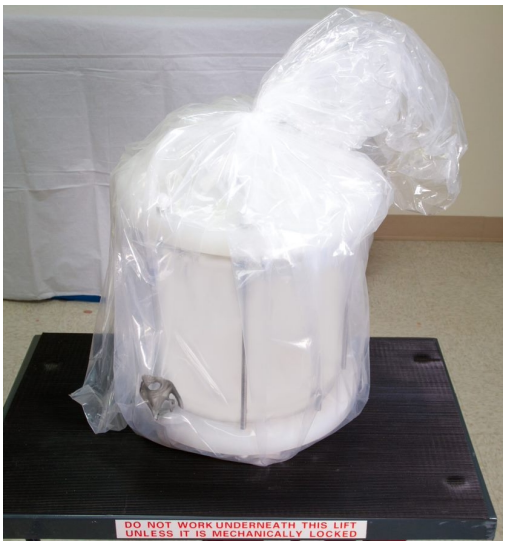
5. Locate and remove the resin reference sample and documents.



6. Remove the empty foam layer to reveal the column wrapped in plastic, and then carefully remove the foam pads.

7. Keeping hands away from the inlet tubing, carefully remove the column from the box by firmly gripping around the edge of the top column plate. A second person can assist with carrying the column.

8. Secure the column in a vertical position and suitable location to avoid any damage.



9. Continue to Removing the Plastic Covers and Zip Ties on page 23.

Removing a Large Column From a Crate

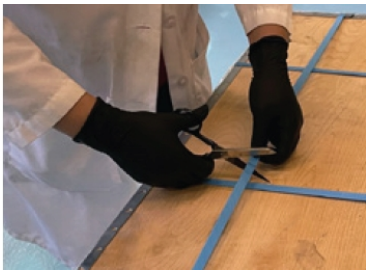
Columns that are 24 cm or 33 cm are shipped in wooden crates that are enclosed in plastic, with foam inserts to protect the contents. This section describes how to open the crate and remove the internal packing and the column without damage.

Important:

- The column is GMP ready. Follow your SOPs for handling GMP ready products.
- These columns are heavy. Use care when handling, and follow your SOP guidelines for handling heavy equipment. Two people to lift are recommended.
- Always secure the column in a vertical position to avoid any damage either in a stationary location or when transporting the column.

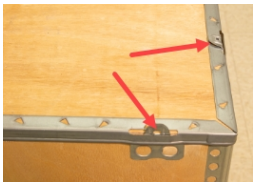
To open the crate and remove the column

1. Inspect the package exterior through the plastic wrap. If you see any damage, If you see any damage, contact Bio-Rad Technical Support at 1-800-424-6723, option 2 or support@bio-rad.com (U.S./Canada Only).
2. Carefully remove the pallet plastic wrap and straps using a cutting tool.

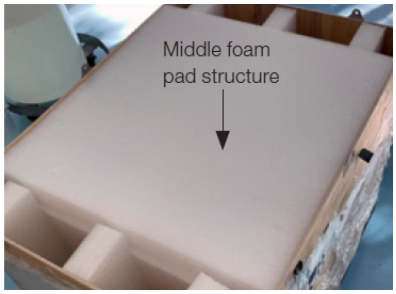


3. Insert a small flathead screwdriver into the central hole of each individual locking pin on the top of the box.

Note: There are ten locking pins, and all must be unlocked before you can remove the lid.



4. Remove the top of the crate, and then remove the foam pad in the middle.

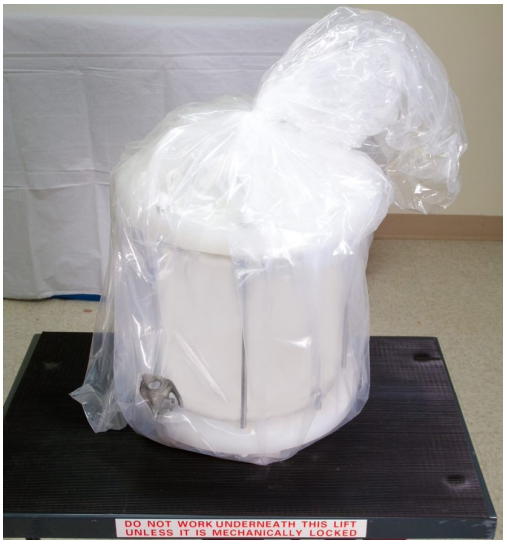


5. Locate and remove the resin reference sample and documents.

6. Locate the tubing and carefully remove the remaining foam pads. Avoid damaging the inlet and outlet tubing when removing the foam.

7. Firmly grip the edge of the top column plate and carefully lift the column straight up to avoid damaging the column and column edges with the plywood box.

8. Secure the column in a vertical position on a suitable surface to avoid any damage either in a stationary location or when transporting the column.



9. Continue to Removing the Plastic Covers and Zip Ties on page 23.

Removing the Plastic Covers and Zip Ties

When you are ready to connect and use the column, complete the instructions in this section to cut and remove zip ties securing the plastic, unwrap the column, and then cut and remove the zip ties and remaining plastic on the column itself.

Important:

- Do not remove the plastic covers and zip ties until you are ready to use the column.
- Foresight Pro columns are GMP ready. Follow your SOP guidelines for handling GMP ready products.
- Use care when cutting to avoid harm to column parts or tubing.
- Use care when lifting and securing the column. Follow your SOPs for lifting and moving heavy objects.
- After removal, dispose of wrappers and zip ties in accordance with local environmental regulations.

Removing Plastic Covers

The column is double bagged and both bags are secured with zip ties.

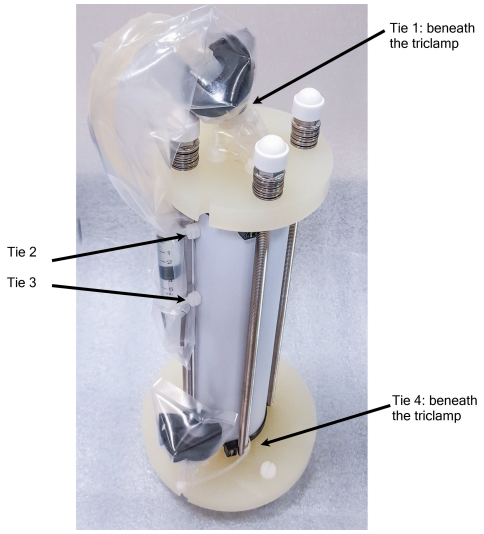
To remove the plastic covers

1. To remove the outer bag, complete the following steps:
    a. Pull the top of the bag up and away from the top of the column and then carefully cut the zip tie.
    b. Slowly pull the bag down until it is below the bottom column plate.
2. Repeat Step 1a and Step 1b to remove the inner bag.
3. Safely lift the column off the bags. Be careful to not damage the inlet and outlet tubing connections.
4. Place the column in the vertical position with the legs on a suitable surface.

Removing Zip Ties on the Column

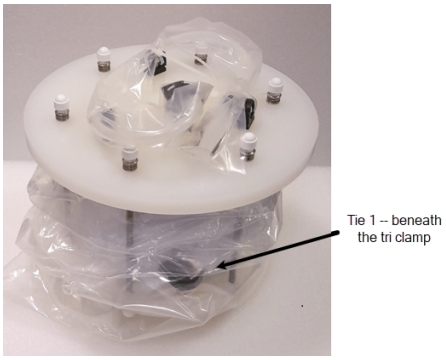
Zip ties and additional plastic wraps are located at different points on the column. Zip ties are placed as follows:

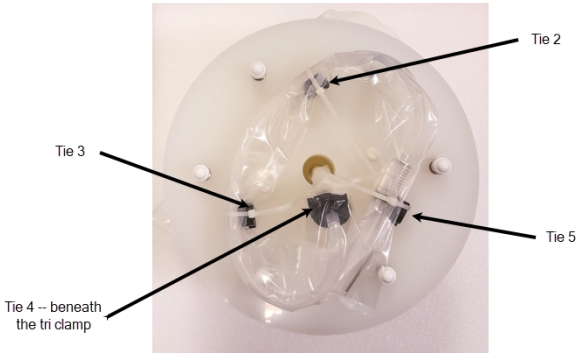
- Columns smaller than 20 cm are sealed with four zip ties, one beneath the Tri-Clamp on the top plate, two on the side, and one beneath the Tri-Clamp on the bottom plate.



- Columns 20 cm and larger are sealed with five zip ties, four on the top plate, beneath the Tri-Clamp and around the tubing, and one on the bottom plate, beneath the Tri-Clamp.

Important: Follow your SOPs for lifting heavy objects. Two people are recommended to lift the heavier columns.





To remove the zip ties

1. Carefully cut and remove each zip tie.

Important: Avoid touching the tubing when you remove the zip ties.

2. Remove the remaining plastic covers on column elements.

Chapter 3 Connecting and Conditioning the Column

After removing the column from the box or crate and securely placing it on the appropriate surface, you can connect the column to your chromatography system.

Note: You can connect to NGC 10 and NGC 100 chromatography systems from Bio-Rad, and AKTA protein purification systems from Cytiva.

Prerequisites

Before connecting the column, complete the following tasks:

1. Set the chromatography system to bypass the column before connection.
2. Start a slow flow based on your setting to prime the system tubing with running buffer, thereby purging existing air bubbles.

Important: Do not exceed 3 bar when operating the column. Refer to your instrument guide for information.

Connecting the Column Inlet

The following graphic and legend identify the components that are typically located on the top plate.

1 Buffer storage tubing

2 Buffer storage tubing seal

3 Tri-Clamp

4 Inlet tubing and seal

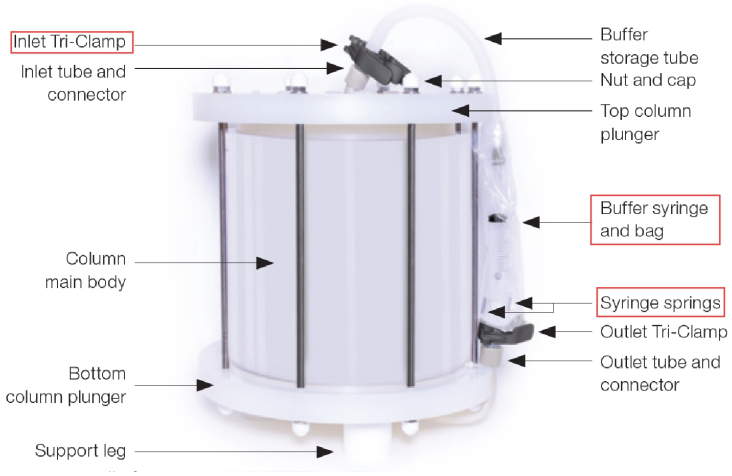
Where a connection is made, ensure that both tubes are filled with liquid. Avoid introducing air into the lines as the connection is sealed.

Note: Spraying all Tri-Clamps with 70% ethanol (EtOH) before making connections is recommended.

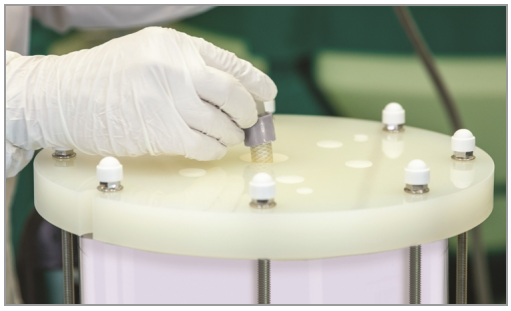
To connect the system to the inlet tubing

1. Ensure the column is placed in the appropriate location near the system.
2. On the top plate of the column, gently extend the flexible buffer storage tubing upwards, and then tap the upper and lower sides at the inlet tubing to allow air bubbles in the tubing to escape.
3. Gently squeeze the buffer storage tubing to prevent drawing air into the inlet tubing.

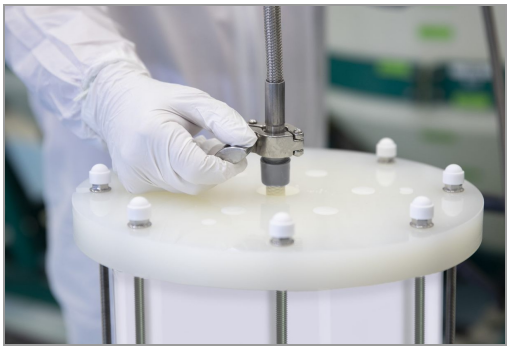
4. To disconnect the top Tri-Clamp, unhook the springs from the top shaft of the syringe and then remove the Tri-Clamp.



5. Remove the buffer storage tubing from the inlet tubing. Avoid trapping air.



6. Tighten the Tri-Clamp.



Connecting the Column Outlet

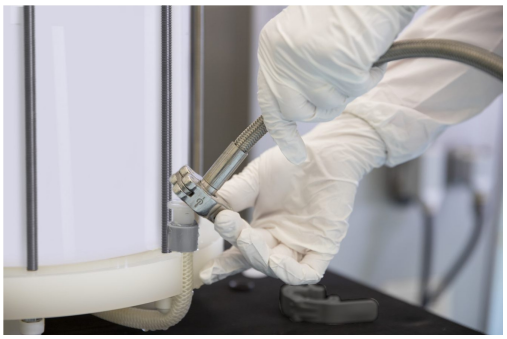
Where a connection is made, ensure that both tubes are filled with liquid. Avoid introducing air into the lines as the connection is sealed.

Note: Spraying all Tri-Clamps with 70% ethanol (EtOH) before making connections is recommended.

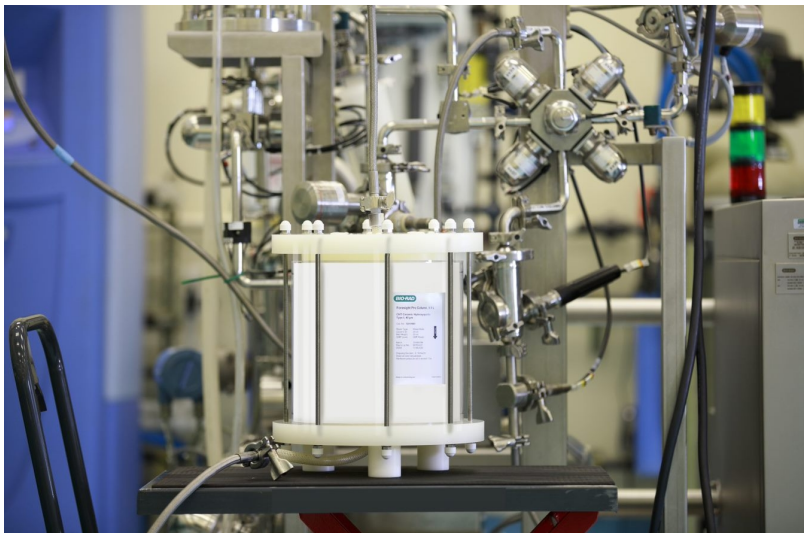
To connect the system tubing to the column outlet

1. Remove the Tri-Clamp and end cap from the outlet on the bottom plate.

2. Connect a Tri-Clamp to the system tubing that connects to the column outlet.



The column is ready for conditioning.



Conditioning the Column

To avoid drying out the bed, ensure one of the following:

- The column remains connected to a buffer reservoir when the column is in use.
- The bottom outlet is sealed.

Removing the Storage Buffer

To remove the storage buffer from the column

▶ Equilibrate the column at a flow rate of 100–200 cm/hr with at least 5 column volumes (CV) of buffer to remove the storage buffer.

Important: To avoid disrupting the packed CHT bed and generating a headspace, operate the column at 3 bar or less.

Chapter 4 Column Sanitization and Storage

When the chromatography process is completed, you can prepare the column for disposal or storage.

Notes:

- Store the Foresight Pro columns in accordance with the procedure cited in Disconnecting and Storing the Column on page 35. For recommendations for the applicable resin, see the resin product information sheet that is shipped with the column.
- Follow your SOPs to clean, flush, and sanitize Foresight Pro columns before disposal.

Cleaning the Column After Use

Refer to your SOPs for information on cleaning the column.

Use any sanitization agent and conditions that are compatible with the materials of construction and chromatography resin. For information on appropriate solvents to use with Foresight Pro columns, see Chemical Resistance Table on page 33.

For detailed compatibility information, see the Ceramic Hydroxyapatite Application Guide.

Important: Do not use alcohols over 20% concentration (v/v) on or with Foresight Pro columns.

Chemical Resistance Table

You can use the information in Table 6 as a reference. Differences in environmental settings, such as temperature, exposure, and other elements might change the product performance. Testing as needed, in the conditions you will use the product, is recommended.

Important: The ratings for the cleaning solvents specified in Table 6 are applicable to ambient temperature conditions only.

The following acronyms apply to the table headings:

PP = polypropylene
EPDM = ethylene propylene diene monomer

The following codes are used to assess chemical resistance to the wetted parts used int he Foresight Pro columns:

E = Excellent
G = Good
F = Fair
P = Poor

Table 6. Chemical resistance for acrylic columns

	Chemical Resistance			
Chemical	**PP**	**Polyamide**	**EPDM**	**Acrylic**
Acetone	E	E	E	P
Acetic acid 5%	E	F	E	G
Ethanol	E	E	E	P (above 20%)
Guanidine	E	E	E	G
Hydrochloric acid (HCl) to pH 2	E	F	E	G
Isopropanol (iPrOH)	E	E	E	P
Methanol (MeOH)	E	F	E	P
Potassium hydroxide (KOH) 1 N	E	G	G	G
KOH 25%	G	F	F	G

Table 6. Chemical resistance for acrylic columns, continued

	Chemical Resistance			
Chemical	**PP**	**Polyamide**	**EPDM**	**Acrylic**
KOH 50%	G	P	F	F
Phosphoric acid (H_3PO_4) to pH 2	E	F	E	G
Sodium hydroxide (NaOH) (0.1 N)	E	G	E	E
Sodium hydroxide (diluted 5%)	E	G	E	G
Sodium hydroxide (25%)	G	F	F	G
Sodium hydroxide (conc 50%)	G	P	F	G
Sodium hydroxide (conc)	G	P	F	G
Urea	E	E	E	F

Disconnecting and Storing the Column

This section contains information for storing your columns.

Important: Before you store a used column, ensure the Tri-Clamps to seal them are sanitized.

To remove a column from a chromatography system

1. Flush the column with the storage liquid.
2. Disconnect the column from the chromatography system and seal the openings with sanitized Tri-Clamp end caps in the following order:
    a. Outlet side
    b. Inlet side
3. Connect one end of the sealing tubing to the inlet tubing of the chromatography system.
4. Hold the sealing tubing upward and run the pump slowly to fill the sealing tubing with the storage liquid.
5. Connect the filled tubing to the inlet, and avoid trapping air bubbles.
6. Disconnect the sealing tubing from the chromatography system.
7. Seal the inlet side with a Tri-Clamp end cap.

The column is ready to be stored.

Refilling the Storage Syringe

Regularly check the buffer level inside the storage syringe.

If the liquid in the syringe barrel is reduced to the following volumes per syringe volume, complete the steps in this section.

- 2 ml in a 5 ml syringe
- 6 ml in a 20 ml syringe
- 12 ml in a 60 ml syringe

To refill the storage syringe

1. Carefully remove the tie and bag from around the syringe.
2. Twist the luer connection to remove the syringe from the storage tubing.

   To avoid letting air into the storage tube, keep the luer end elevated.
3. Disconnect the springs from the top of the syringe plunger.
4. Fill the syringe barrel with storage liquid to the levels specified below.

Syringe volume	Fill to this volume
5 ml for 5 cm and 8 cm inner diameter	5 ml
20 ml for 10 cm and 13 cm inner diameter	14 ml
20 ml for 20 cm inner diameter	17 ml
20 ml for 24 cm inner diameter	20 ml
60 ml for 33 cm inner diameter	35 ml

5. Reconnect the syringe to the storage tubing via the luer connection.
6. Reconnect the springs to the top of the syringe plunger

   Important: Do not overstretch the springs.
7. Repeat Steps 1 to 5 at regular intervals (typically every 2-3 months).

Disposal

Sanitize the used Foresight Pro columns before disposal, in accordance with the information in Column Sanitization and Storage on page 32 and local regulations.

Appendix A Column Troubleshooting

Troubleshooting For CHT Columns

Table 7 section provides information required to enable users to identify and correct problems that may occur when operating Biotoolomics disposable columns.

If the suggested actions in this guide do not solve the problem, or if the problem is not covered by this guide, contact Bio-Rad Technical Support at 1-800-424-6723, option 2. For more information see Bio-Rad Technical Support Department on page 2.

Important: Do not operate the column in a reverse flow direction.

Table 7. Troubleshooting issues and corrective actions

Issue	Possible cause	Corrective action
Air has entered into the column	During the installation or during a run	Follow the steps in Removing Air From the Column Bed on page 40.
High backpressure	■ The column is clogged during run ■ Incompatible buffer or pH applied to the column	Do the following: 1. Perform regeneration of the column. 2. Filter the sample before it is applied. 3. Follow the resin instruction to choose suitable buffer systems.

Table 7. Troubleshooting issues and corrective actions, continued

Issue	Possible cause	Corrective action
Efficiency test results are not acceptable	■ Check for extra column effects (large or overly long tubing)	■ Ensure the sample is not being diluted before application to the column. ■ Position the detector as close to the column outlet as possible
	■ The column might not be equilibrated	■ Ensure the column is fully equilibrated to stable pH and conductivity
	■ Incorrect type of buffer and/or tracer used	■ Ensure sample running buffer are the same as used for QA release testing.
Efficiency test result is not the same as on the certificate	The test is performed on a different instrument than the one used in the production. **Note:** Each test system and method used will contribute to the test result and variance.	■ If the test result is within acceptance limits, the column is acceptable to use. ■ Ensure size and concentration of the sample are the same as used for the QA release test.

Removing Air From the Column Bed

The following methods are suggested to remove air from the column bed.

Method 1

This method is recommended if air is trapped at the bottom part of the column.

1. Install a fully open flow restriction valve downstream, on the column outlet.
2. Apply a low flow velocity (for example 30 cm/h) through the column.
3. Restrict the flow through the outlet restrictor valve, so that a backpressure of approximately 2-3 bar is applied over the column bed.
4. Run the column for 5 column volumes (5 CV).
5. Run an efficiency test on the column to evaluate air removal. If air is still present, repeat this procedure.

Method 2

This method is recommended if air is trapped at the top part of the column.

1. Install a three-way valve at the column inlet, allowing liquid inside the column to go backwards in two directions. Connect one port to the pump, and connect the other port to a waste bottle using a suitable tubing.
2. Close the outlet.
3. Apply a low flow velocity (for example 30 cm/h) through the column at disdainful direction so that a back pressure of approximately 2-3 bar is built over the column bed.
4. Quickly switch the valve to the waste bottle direction, allowing air to rapidly burst out.
5. Repeat steps 3 and 4 several times.
6. Run an efficiency test on the column to evaluate air removal.

Method 3

This method is recommended if air is trapped at the resin bed

1. Keep the outlet fully open to a suitable waste container.
2. Apply a high flow velocity (for example 250 cm/h) through the column.
3. Run the column for 5 column volumes (5 CV).
4. Run an efficiency test on the column to evaluate air removal. If air is still present, repeat this procedure or try Method 1.

Appendix B Column Validation Data

Packing Quality

The performance of Foresight Pro columns was evaluated before and after International Safe Transit Association (ISTA) 2A shipping tests. The column efficiency test includes the determination of the number of theoretical plates per meter (N/m) and asymmetry factor (A_s).

Note the following:

- The Foresight Pro columns were equilibrated in phosphate buffered saline (PBS), pH 7.2.
- Each column was injected with a 2% column volume of PBS containing 1.0 M NaCl.
- The flow rate was continued at 100 cm/hr while monitoring conductivity.
- N/m and A_s values were then determined. As shown in Fig. 1 and Fig. 2 on page 43, the columns maintained performance criteria (Table 8) with minimal changes in both N/m and A_s, indicating good stability during shipping.

Table 8. Packed column specifications

Criteria	Value
Number of theoretical plates per meter (N/m)	$\geq$ 4800
Column asymmetry.(A_s)	$1.80 \leq A_s \leq 2.2$

Fig. 1: Evaluation of N/m, comparing pre-ISTA 2A tests to post-ISTA 2A tests

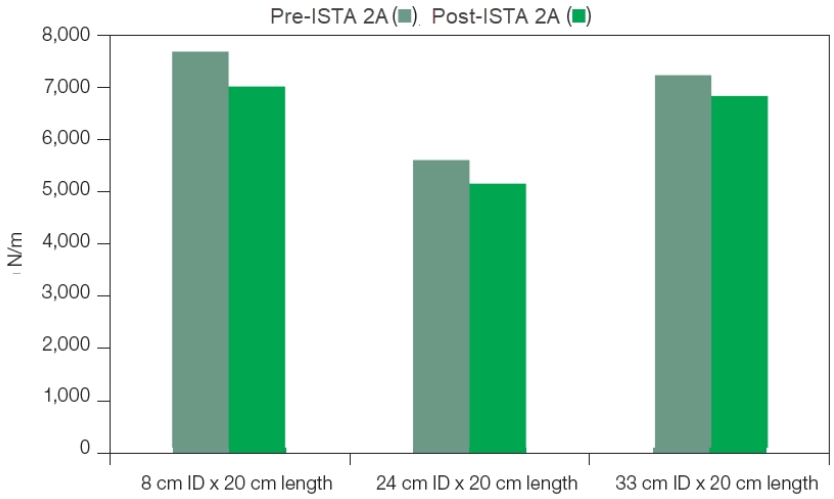
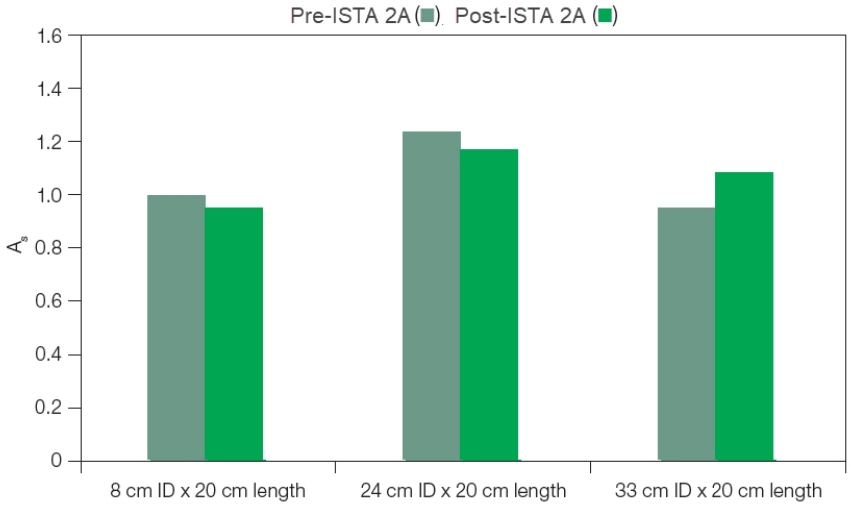


Fig. 2: Evaluation of A_s, comparing pre-ISTA 2A tests to post-ISTA 2A tests



Cycling Data

Testing for flow stability is a further assurance that the column beds are stable. For this study, packed columns were put through ten cycles of increasing flow rates, in increments of 50 cm/hr up to a backpressure of 3 bar. Each flow rate was held for 2 min, and the process was repeated ten times.

Column efficiency was measured in terms of N/m and A_s prior to and at the end of the cycling study. All columns maintained performance criteria after the tenth cycle. As shown in Fig. 3 and Fig. 4, changes in column efficiency were minimal.

Table 9. Packed column specifications

Criteria	Value
Number of theoretical plates per meter (N/m)	$\geq$ 4800
Column asymmetry.(A_s)	$1.80 \leq A_s \leq 2.2$

Fig. 3: Evaluation of N/m, comparing the first and tenth cycle of three Foresight Pro columns

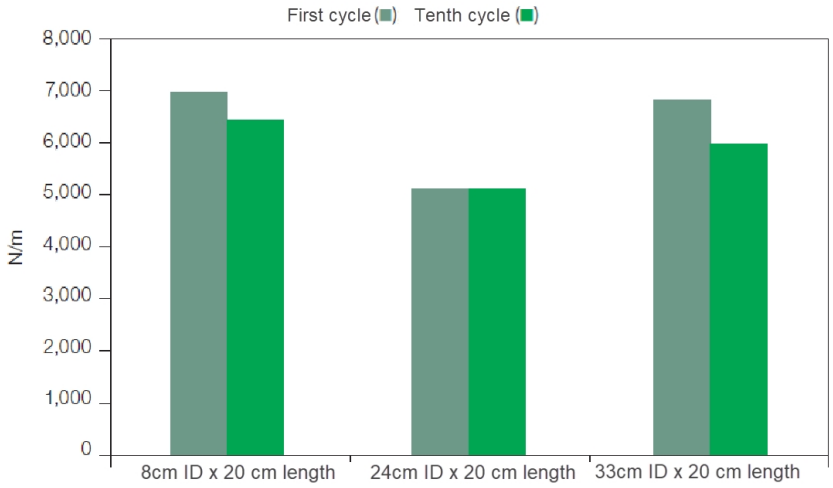
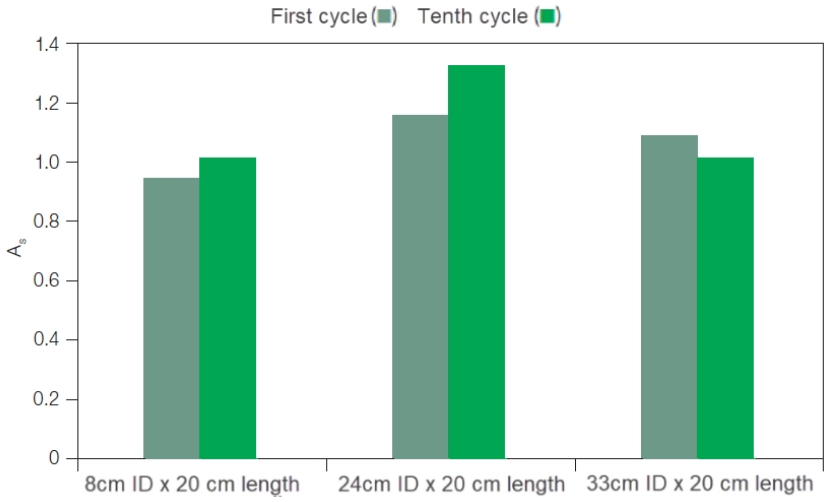


Fig. 4: Evaluation of A_S, comparing the first and tenth cycle of three Foresight Pro columns



CIP Efficiency

Both endotoxin and bioburden testing are performed to ensure that columns are in a sanitized state for shipment to customers. Each column must pass the acceptance criteria for endotoxin level and microbial bioburden as shown in Table 10 below.

Table 10. Bioburden and endotoxin levels

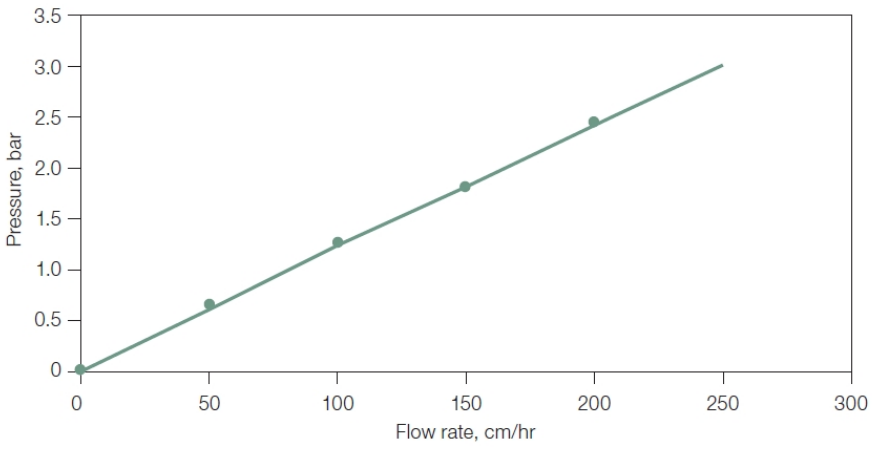
Criteria	Value
Microbial bioburden, CFU/mL	< 10 CFU/ml
Endotoxin level, EU/ml	< 0.25 CFU/ml

Pressure/Flow Performance

Fig. 5 shows an example of the linear relationship between pressure and flow rate up to 250 cm/hr on Foresight Pro columns packed with CHT Type I. The data indicates that the column pressure remains below 3 bar at a linear velocity of 250 cm/hr.

Note: The Foresight Pro columns were equilibrated in phosphate-buffered saline (PBS), pH 7.2.

Fig. 5: Pressure/flow performance, packed with CHT Ceramic Hydroxyapatite Type I, 1 L, 8 cm x 20 cm



Column Scale-up Study

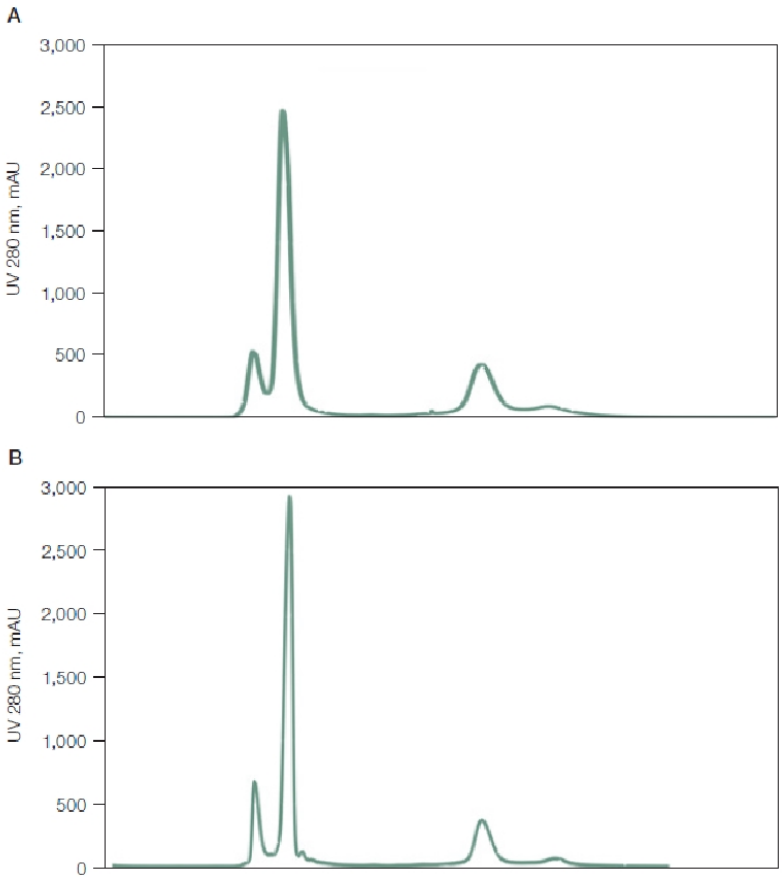
A study was performed to confirm that the same purification results are obtained irrespective of the prepacked column size. This study successfully demonstrated highly similar results as indicated by the similar elution profile on each chromatogram.

Note the following:

- A laboratory column (1.5 cm inner diameter x 20 cm length) was compared to a Foresight Pro Column (8 cm inner diameter x 20 cm length).
- Each column used in the study was prepacked with CHT Type I Media (40 µm particle size).
- A sample mixture containing bovine serum albumin, lysozyme, and lactoferrin was loaded to 0.8–1 mg/ml bed volume.
- A buffer of 5 mm sodium phosphate and 10 mm NaCl, pH 7.2, was used for equilibration.
- The sample mixture was eluted at 100 cm/hr in a linear gradient of 5 mm sodium phosphate, 10 mm NaCl,
- pH 7.2 to 400 mm sodium phosphate, pH 7.2, over 10 CV, followed by a 3 CV hold in the high phosphate buffer.

As shown in Fig. 6 on page 48, this study successfully demonstrated the same purification results as indicated with the similar elution profile on each chromatogram.

Fig. 6: Comparative purification results of A, laboratory column CHT Type 1, 40 µm, 1.5 x 20 cm and B. CHT Type 1, 40 µm, 5 x 20 cm



Column Shelf Life

All Foresight Pro columns have a shelf life of two years from the date of manufacture.

Appendix C Glossary of Terms

The following acronyms and units of measure or value are used in this document.

Term	Definition
A_s	Asymetrical diameter
CFR	Code of Federal Regulations
CFU	Colony-forming unit
CHT	Ceramic Hydroxyapatite Type
cm	Centimeter
CV	Column volume
FDA	U.S. Food and Drug Administration
EMEA	Europe, Middle East, Africa
EPDM	Ethylene propylene diene monomer rubber
EtOH	Ethanol
EU	Endotoxin units
FDA	U.S. Food and Drug Administration
GMP	Good Manufacturing Practices
HCl	Hydrochloric acid
H_3PO_4	Phosphoric acid
Hr	Hour
ID	Inner diameter
iPrOH	Isopropanol
ISTA	International Safe Transit Association

Term	Definition
KOH	Potassium hydroxide
MeOH	Methanol
mm	Millimeter
NaOH	Sodium hydroxide
N/m	Number of theoretical plates per meter
PBS	Phosphate-buffered saline
PP	Polypropylene
SOP	Standard Operating Procedure
USP	U.S. Pharmacopeia
v/v	Volume-to-volume
µm	Micrometer

Appendix D Ordering Information

This section contains tables of product information for Foresight Pro prepacked columns with specified resins. Appendix D contains product information for prepacked columns that are available to consumers.

Table 11. Prepacked columns

Catalog Number	Product Description	Diameter (cm)	Length (cm)	Volume (L)
12014659	Foresight Pro Column, CHT Type I, 40 µm, 0.2 L	5	10	0.2
12014660	Foresight Pro Column, CHT Type I, 40 µm, 0.4 L	5	20	0.4
12014671	Foresight Pro Column, CHT Type I, 40 µm, 0.5 L	8	10	0.5
12014672	Foresight Pro Column, CHT Type I, 40 µm, 1.0 L	8	20	1
12014673	Foresight Pro Column, CHT Type I, 40 µm, 0.8 L	10	10	0.8
12014674	Foresight Pro Column, CHT Type I, 40 µm, 1.5 L	10	20	1.5
12014675	Foresight Pro Column, CHT Type I, 40 µm, 1.3 L	13	10	1.3
12014676	Foresight Pro Column, CHT Type I, 40 µm, 2.7 L	13	20	2.7
12014677	Foresight Pro Column, CHT Type I, 40 µm, 3.1 L	20	10	3.0
12014678	Foresight Pro Column, CHT Type I, 40 µm, 6.3 L	20	20	6.3
12014679	Foresight Pro Column, CHT Type I, 40 µm, 4.5 L	24	10	4.5
12014680	Foresight Pro Column, CHT Type I, 40 µm, 9.0 L	24	20	9
12014681	Foresight Pro Column, CHT Type I, 40 µm, 8.5 L	33	10	8.5
12014682	Foresight Pro Column, CHT Type I, 40 µm, 17 L	33	20	17
12014697	Foresight Pro Column, CHT Type II, 40 µm, 0.2 L	5	10	.2
12014698	Foresight Pro Column, CHT Type II, 40 µm, 0.4 L	5	20	0.4

Table 11. Prepacked columns

Catalog Number	Product Description	Diameter (cm)	Length (cm)	Volume (L)
12014699	Foresight Pro Column, CHT Type II, 40 µm, 0.5 L	8	10	0.5
12014700	Foresight Pro Column, CHT Type II, 40 µm, 1.0 L	8	20	1
12014701	Foresight Pro Column, CHT Type II, 40 µm, 0.8 L	10	10	0.8
12014702	Foresight Pro Column, CHT Type II, 40 µm, 1.5 L	10	20	1.5
12014703	Foresight Pro Column, CHT Type II, 40 µm, 1.3 L	13	10	1.3
12014704	Foresight Pro Column, CHT Type II, 40 µm, 2.7 L	13	20	2.7
12014705	Foresight Pro Column, CHT Type II, 40 µm, 3.1 L	20	10	3.1
12014706	Foresight Pro Column, CHT Type II, 40 µm, 6.3 L	20	20	6.3
12014707	Foresight Pro Column, CHT Type II, 40 µm, 4.5 L	24	10	4.5
12014708	Foresight Pro Column, CHT Type II, 40 µm, 9.0 L	24	20	9
12014709	Foresight Pro Column, CHT Type II, 40 µm, 8.5 L	33	10	8.5
12014710	Foresight Pro Column, CHT Type II, 40 µm, 17 L	33	20	17
12014725	Foresight Pro Column, CHT XT, 40 µm, 0.2L	5	10	0.2
12014726	Foresight Pro Column, CHT XT, 40 µm, 0.4 L	5	20	0.4
12014727	Foresight Pro Column, CHT XT, 40 µm, 0.5 L	8	10	0.5
12014728	Foresight Pro Column, CHT XT, 40 µm, 0.2 L	8	20	1
12014729	Foresight Pro Column, CHT XT, 40 µm, 1.0 L	10	10	0.8
12014730	Foresight Pro Column, CHT XT, 40 µm, 1.6 L	10	20	1.6
12014731	Foresight Pro Column, CHT XT, 40 µm, 1.3 L	13	10	1.3
12014732	Foresight Pro Column, CHT XT, 40 µm, 2.7 L	13	20	2.7
12014733	Foresight Pro Column, CHT XT, 40 µm, 3.1 L	20	10	3.1

Table 11. Prepacked columns

Catalog Number	Product Description	Diameter (cm)	Length (cm)	Volume (L)
12014734	Foresight Pro Column, CHT XT, 40 µm, 6.3 L	20	20	6.3
12014735	Foresight Pro Column, CHT XT, 40 µm, 4.5 L	24	10	4.5
12014736	Foresight Pro Column, CHT XT, 40 µm, 9.0 L	24	20	9
12014737	Foresight Pro Column, CHT XT, 40 µm, 8.5 L	33	10	8.5
12014738	Foresight Pro Column, CHT XT, 40 µm, 17 L	33	20	17

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