

OPUS[®] 2.5 - 8 Pre-packed Chromatography Columns

USER GUIDE

For use with:

- OPUS[®] 2.5 Pre-packed Chromatography Columns
- OPUS[®] 5 Pre-packed Chromatography Columns
- OPUS[®] 8 Pre-packed Chromatography Columns



OPUS[®] 2.5



OPUS[®] 5



OPUS[®] 8

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Abbreviations

A _s	Peak asymmetry
ABS	Acrylonitrile butadiene styrene
BSE	Bovine spongiform encephalopathy
CFR	Code of Federal Regulations
CoA	Certificate of Analysis
CoQ	Certificate of Quality
CV	Column volume
GMP	Good manufacturing practice
HETP	Height equivalent to a theoretical plate
PPE	Personal protective equipment
N	Number of theoretical plates
OPUS®	Open Platform User Specified
PBS	Phosphate buffered saline
PP	Polypropylene
PCS	Platinum cured silicone
QC	Quality Control
RSF	Regulatory Support File
TSE	Transmissible spongiform encephalopathy
USP	United States Pharmacopeia
V _e	Peak elution distance
W _{1/2}	Peak width at half height

1. About this document

This manual uses several different phrases. Each phrase should draw the following level of attention:

Table 1. Explanation of user attention phrases

Phrase	Description
Note:	Points out useful information.
IMPORTANT	Indicates information necessary for proper instrument operation.
PRECAUTION	Cautions users of potential physical injury or equipment damage if the information is not heeded.
WARNING!	Warns users that serious physical injury can result if warning precautions are not heeded.

2. Safety precautions

Table 2. Safety precautions for OPUS® 2.5 – 8 Pre-packed chromatography columns

Symbol	Description
WARNING 	Wear standard laboratory personal protective equipment (PPE), including lab coat, protective eye wear, and gloves.
WARNING 	This product is for laboratory and manufacturing production use only. Not for administration to humans.
IMPORTANT 	Unless otherwise specified by the end-user, OPUS® pre-packed columns are generally shipped in 20% ethanol solution, a recognized bacteriostatic agent. Consult the Certificate of Analysis (CoA) or Certificate of Quality (CoQ) for confirmation of storage solution.
WARNING 	Flammable liquid and vapor. <ul style="list-style-type: none"> • Keep away from heat/spark/open flame/hot surfaces. No smoking. • Keep container tightly closed. • Ground/bond container and receiving equipment. • Store in a well-ventilated place. Keep cool.
IMPORTANT 	Dispose of contents/container in accordance with local/regional/national/international regulations.
IMPORTANT 	Removing the column from shipping materials: Use two hands to grab the column body and lift from the foam inserts within the packaging box.
IMPORTANT 	Do not pull or strain the white inlet and outlet ports protruding from the top of the OPUS® column.
IMPORTANT 	For a full list of precautionary statements, please review the Regulatory Support File (RSF).

3. Introduction

This user guide provides general guidance for the use of OPUS® 2.5 – 8 Columns. For further optimization or troubleshooting support, please contact the Repligen Customer Service team (email: customerserviceUS@repligen.com; phone: 781-250-0111).

For additional data supporting the design of the OPUS® product line and testing of qualified processes, refer to the Regulatory Support File (RSF). The RSF may be accessed via the Repligen website.

3.1 What are Open Platform User Specified (OPUS®) Columns?

Open Platform User Specified (OPUS®) Columns are designed to perform chromatography purification of biological molecules in either GMP or non-GMP applications.

The OPUS® Pre-Packed Column platform offers an alternative to conventional “pack in place” glass or stainless steel columns and can be reliably packed with virtually any resin from any source. To accommodate a wide range of biopharmaceutical applications, OPUS® Columns are configurable for nearly any bed height and industry standard internal diameters.

3.2 Column design

The OPUS® platform has been designed to meet the requirements of GMP manufacturing in the pharmaceutical and biopharmaceutical industries for campaign-use and single-use applications.

3.3 Platformable

OPUS® Columns are designed to be broadly configurable to accommodate a wide range of purification and polishing applications for vaccines, monoclonal antibodies, and recombinant proteins. For example, OPUS® Columns are:

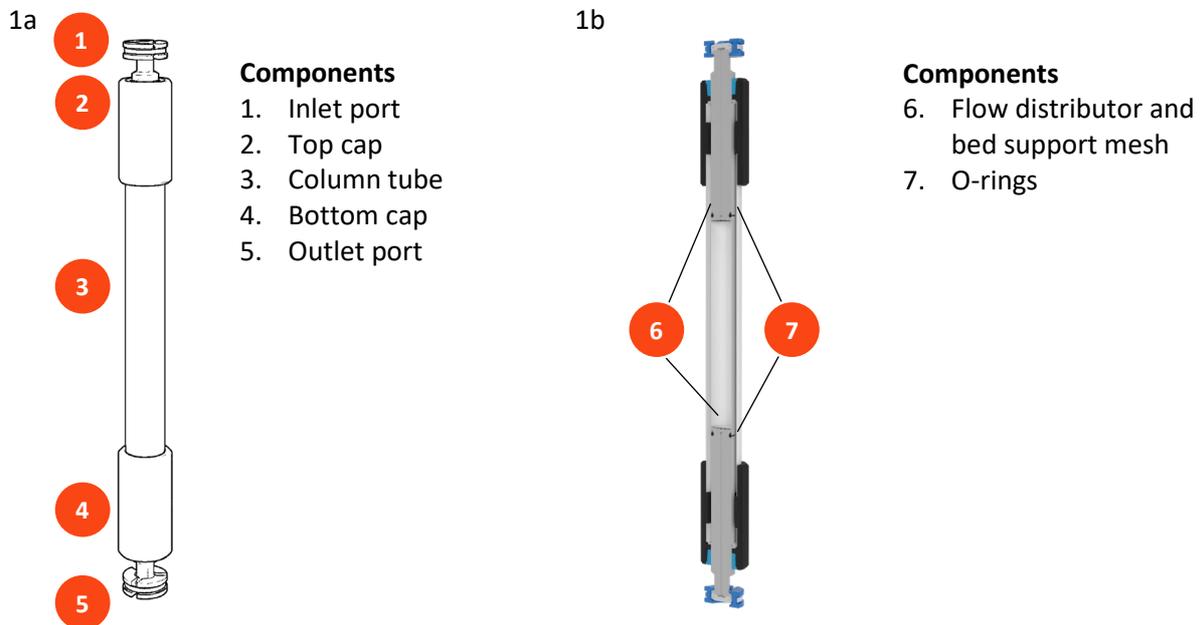
- Compatible with nearly all commercially available bioprocessing resins.
- Available in a wide range of bed heights and industry standard column diameters.
- Configurable for specific packing procedures, release tests, and storage solutions.

4. Column components and materials of construction

4.1 Column components

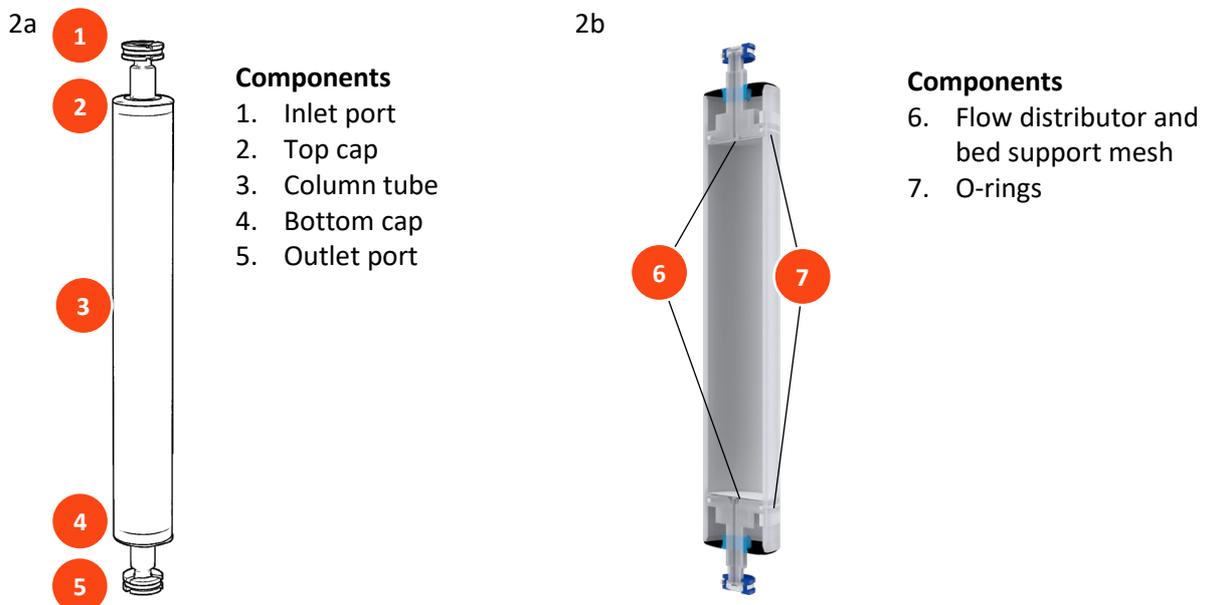
OPUS® 2.5 Column components are labeled below ([Figure 1](#)): external view (1a), cutaway view (1b).

Figure 1. OPUS® 2.5 Column components



OPUS® 5 and OPUS® 8 Column components are labeled below ([Figure 2](#)): external view (2a), cutaway view (2b).

Figure 2. OPUS® 5 and 8 Column components



4.2 Column materials of construction

OPUS® 2.5 - 8 Columns are manufactured using polymers compatible with bioprocess requirements (Table 3).

Table 3. Product materials and quality standards

Component	Material	USP	CFR 21 177	BSE/TSE
Inlet/Outlet port	Compression molded PP homopolymer	Class VI USP <88>	177.1520	Animal Free
Top/Bottom cap	ABS	N/A, non-product contacting		
Flow distributor	Compression molded PP homopolymer	Class VI USP <88>	177.1520	Animal Free
Bed support mesh	PP woven mesh	Class VI USP<88>	177.1520	EMA 410/01
Column tube	Extruded PP homopolymer	Class VI USP <88>	177.1520	Animal Free
O-rings	PCS	Class VI	177.2600	Animal Free
Port gaskets	PCS	Class VI	177.2600	Animal Free

PP = Polypropylene, PCS = Platinum cured silicone, ABS = Acrylonitrile butadiene styrene.

4.3 OPUS® Column physical specifications

Table 4. Physical specifications summary

Physical attribute	Column diameter		
	2.5 cm	5.1 cm	8.1 cm
Internal cross-sectional area	4.91 cm ²	20.4 cm ²	51.5 cm ²
Column body pressure rating	4 bar	4 bar	4 bar
Bed height range	5 - 30 cm	5 – 30 cm	5 – 30 cm
Column volumes			
10 cm bed height	0.05 L	0.2 L	0.5 L
20 cm bed height	0.10 L	0.4 L	1.0 L
30 cm bed height	0.15 L	0.6 L	1.5 L
Assembled column height	~25 cm + bed height	~20 cm + bed height	~20 cm + bed height
Outer diameter (including caps)	6 cm	7 cm	10 cm
Inlet/outlet flow path internal diameter	2.00 mm 5/64 inch	3.45 mm 5/32 inch	4.57 mm 3/16 inch
Inlet/outlet port connectors	3/4 inch mini tri-clamp	3/4 inch mini tri-clamp	3/4 inch mini tri-clamp

4.4 Solvent compatibility

The product contact materials of construction consist of polypropylene and silicone parts. [Table 5](#) lists common solutions with excellent compatibility with both polypropylene and silicone.

Table 5. Solvent compatibility for OPUS® Columns

Water	Citric acid
20% acetic acid	Methanol
20% ethanol	Phosphoric acid
10% acetone	Hydrochloric acid (<20%)
2% (w/v) detergents	Sulfuric acid (<50%)
8 M urea or 6 M guanidine HCl	2 M sodium hydroxide
Potassium hydroxide	Benzyl alcohol
Isopropyl alcohol (IPA)	

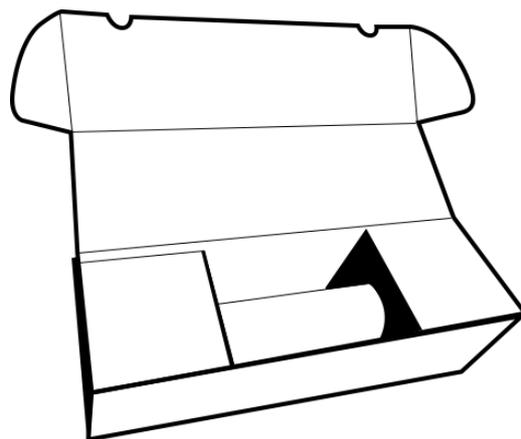
5. Preparing to use the OPUS® Column

1. Upon receipt of the box:
 - a. Inspect the outside of the heavy-duty cardboard carton for any unusual signs of damage. If significant damage has occurred, please contact Repligen Customer Service immediately with pictorial evidence.
 - b. Locate the shipping delivery documents attached to the outside of the box.
 - c. Locate the Certificate of Analysis (CoA) for the column inside the box.
 - d. Unfold the top flaps of the shipping over-box to reveal the set of individual white product boxes.
 - e. For GMP Run Ready OPUS® Columns, locate the QC resin sample and store as specified by the resin supplier.
 - f. In some cases, excess resin not used during column packing may also be shipped along with the column box.
2. Remove the individual product boxes from the shipping over-box. Follow the process outlined below to open the individual product box for each OPUS® Column ([Figure 3](#)).
 - a. Position the product box such that the longest face is parallel to the unpackaging table/bench.
 - b. Unfold the top flap of the individual product box.
 - c. Slide the foam inserts and the column out of the box as one cohesive unit.
 - d. For maximum cleanliness, keep the clear plastic bag containing the column intact for this step.

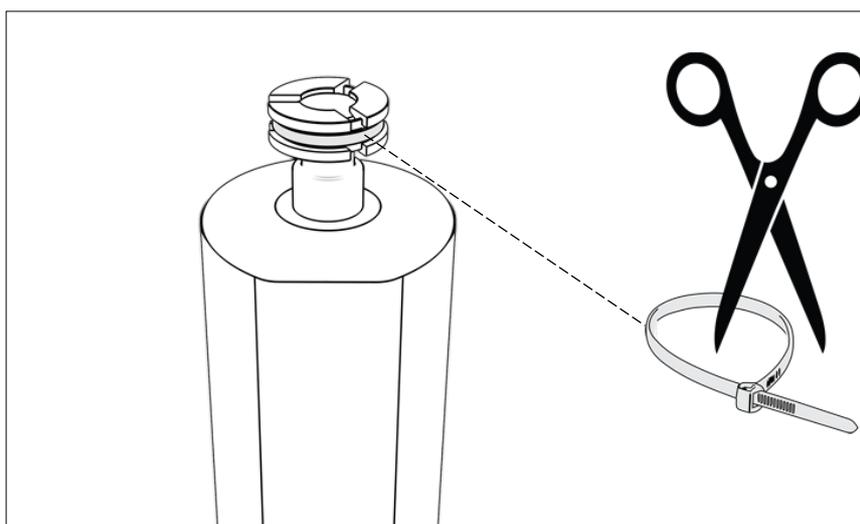


IMPORTANT: Do not pull or strain the white inlet and outlet ports protruding from each end of the OPUS® Column.

3. If you are not ready to use the column, refer to the storage recommendations of the resin supplier for the pre-packed resin.
 - a. Storage conditions are also suggested on the OPUS® Column label and CoA report.

Figure 3. OPUS® Column shipping box and foam insert

4. When you are ready to use the column, remove it from the clear plastic bag. Wipe down the column's exterior surfaces with ethanol or isopropyl alcohol solution as needed.
 - a. If the column was stored at 2 - 8° C, allow the column to equilibrate to room temperature for ≥ 4 hours prior to use of the column.
 - b. Use a wire cutter or sharp scissors to remove the white cable-tie on the SaniSure® clamp sealing the inlet port ([Figure 4](#)).

Figure 4. SaniSure® clamp removal

5. Reference the technical specifications below when using your OPUS® Column during processing:
 - a. Minimum packing pressure: Chromatography system pressure alarms should be set based on the packing pressure for your particular OPUS® Column.

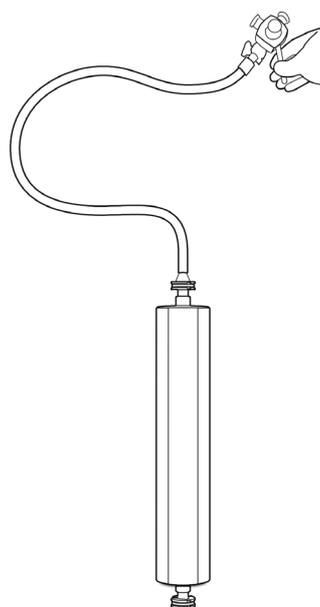
Note: Maximum pressure achieved during packing is noted on either the column label and/or CoA. Exceeding the maximum pressure achieved during packing will likely compromise the packed bed integrity and void the Repligen warranty statement (see Section 2.5 of the [OPUS® Columns Regulatory Support File](#)).

- b. Temperature: Column construction is designed to support a working temperature range of 2 - 40° C.

5.1 Connecting and operating the OPUS® Column

- **Use of stainless steel valves connected directly to the inlet and/or outlet ports is not recommended. The additional weight to the top of the column will increase the risk of damage to the column hardware.**
- If valves must be used, first connect the inlet and outlet ports to tubing. Then, the other end of that tubing may be connected to valves ([Figure 5](#)).
- A tubing length of < 25 cm is suggested to minimize hold up volume of the system.

Figure 5. Stainless steel valve connection to OPUS® Column



- If an OPUS® Column needs to be connected to a chromatography system that utilizes threaded components, an adapter will be needed (25 mm TC – UNF 5/16” Female).
 - Instructions for using a three-way valve to connect the column inlet and to purge air from the inlet line can be found in [Appendix 1](#).
 - Instructions on how to connect an OPUS® Column to a chromatography system can be found in [Appendix 2](#).
1. Connect the column to the chromatography system while limiting the entry of air to the inlet port connection. Repligen suggests the following sequence for reducing air introduction to the column:
 - a. Top off the inlet port with low salt equilibration buffer (e.g. 0.1 M NaCl) prior to making connections if needed.
 - b. Hook up the column inlet under low flow (~50 cm/hr) with a tri-clamp so that no air is introduced into the column.
 - c. Once the inlet has been connected under low flow, connect outlet tubing to the outlet port and flush the storage solution from the column with 3-5 column volumes of low buffer (e.g. PBS).
 - d. All solutions loaded on to the column should be 0.22 or 0.45 µm filtered to reduce column fouling.
 - e. Start storage solution flush with 50 cm/hr flow.
 - f. Flow rate may be increased as needed while maintaining conformance to the maximum packing pressure reported on the column CoA.

Note: The column may have off-gassed during shipment which leaves the top port dry; however, the packed bed will remain hydrated in its storage solution.

Note: High salt solutions should only be used after column is equilibrated with a low salt solution. When in contact with ethanol solutions, high salt concentration buffers may precipitate solids into the packed bed.

Note: While flushing the ethanol storage solution out of the column, high pressures are to be expected due to the viscosity of the solution. Flow rate must be reduced if pressures approach 75% of the column packing pressure.

2. Equilibrate your column using 2-3 CVs of your equilibration process buffer or mobile phase. Perform a slow ramp up of flow rate to 50-75% of the packing pressure to sufficient flush the column of entrapped air.
 - a. If performance of the column is non-conforming, consult [Section 7.1](#).
3. To test for chromatographic performance and compare results to the CoA, a short instruction guide can be found in [Appendix 3](#).

6. OPUS® Column sanitization, storage, and disposal

- Please consult the resin supplier for recommended cleaning and storage protocols.
- OPUS® Columns can be cleaned with any sanitization agent that is compatible with the materials of construction (see [Table 5](#) for Solvent Compatibility Summary).
- Prior to sanitizing your column, check for solvent compatibility with the chromatography resin supplier.
 1. Once your chromatography process is completed, the column should be prepped for disposal or storage:
 - Disposal: Clean and sanitize the column prior to disposal according to local government regulations.
 - Storage: Clean, flush, and prepare the column for storage per the recommendations of the resin supplier or other validated procedure.
 2. To re-use the OPUS® Column post storage: Start with the general usage instructions in [Section 5.1](#), “Connecting and operating the OPUS® Column”.

7. Troubleshooting

7.1 Air in the column

Causes

- Dissolved air within the storage solution may degas out of solution after the shipping process. Pressure and temperature fluctuations during storage/transit may release the dissolved air and allow aggregation at the top of the OPUS® Column flow ports.
- Internal diameter of flow path is too large for the associated OPUS® Column diameter.
- Insufficient priming of the flow path tubing before connection to the OPUS® Column.
- Pump introduces air into flow path as buffer is depleted.

Potential Fixes

- If air entered the inlet port and did not reach the packed bed within the column (to the best assessment of the operator), follow the air purge procedure described in [Appendix 1](#).
- If air entered the packed chromatography bed, recondition the column by operating column in reverse flow for 2-3 CVs. Increased backpressure on the column effluent may aid in

forcing air bubbles out from the column. However, differential pressure should not exceed the maximum packing pressure reported on the column CoA.

- Contact Repligen Customer Support for troubleshooting tactics specific to column dimension and packed resin.
- Re-test column performance (efficiency, asymmetry) according to instructions in [Appendix 3](#). Results conforming to the provided CoA for your column will help justify release into production.

7.2 High pressure during first use of column

Causes

- Undersized tubing, fitting, and/or gaskets.
- Incorrect column valve position.
- Flow path restriction.
- Operation under higher flow rate than recommended for the packed resin bed.
- Temperature shifts between buffers used in the column.

Potential Fixes

- Refer to [Table 4](#) for flow path sizing.
- Ensure valve is in the proper position.
- Reduce the flow rate of solution through the column to abide by pressure limit of the packed resin bed.
- Confirm that high viscosity solutions are not being used during pressure evaluation.
- Flow of alcohols through the column is known to increase column pressure.
- Allow all buffers and the column to equilibrate to ambient temperatures.

7.3 Pressure increase during run

Causes

- Product or precipitates clogging the polypropylene mesh.
- Operating with flow rate that is higher than recommended for the packed resin.
- Residue build up at the top of the column.
- Use of high viscosity solutions or high product load concentrations.
- Fouled chromatography resin.
- Temperature shifts between buffers used in the column.

Potential Fixes

- Clean the column with the appropriate cleaning method for the residue that clogged the mesh and/or resin. Running in reverse flow or up-flow mode is recommended.
- Flow > 5 CV of equilibration buffer through the column in reverse flow. Re-check pressure and column performance (efficiency, asymmetry, pressure vs. flow) under normal operating conditions; compare to CoA.
- Ensure valve is in the proper position.
- Allow all buffers and the column to equilibrate to ambient temperatures.

7.4 Pressure drop during run

Causes

- Line or fitting leaks
- Temperature shifts during buffer transitions
- Viscosity shifts during buffer transitions

Potential Fixes

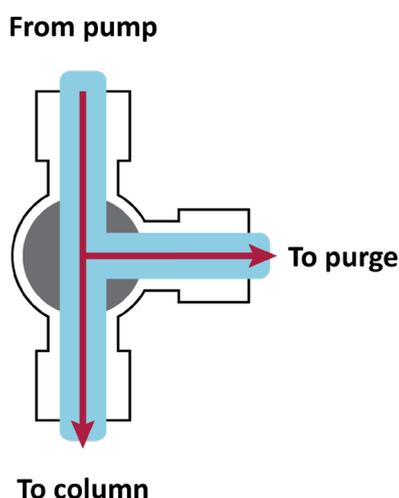
- Check lines and connections
- Allow all buffers and the column to equilibrate to ambient temperatures

8. Appendices**8.1 Appendix 1: Use of a three-way valve for inlet connection and air purging**

To purge air when the column is first connected to a chromatography system:

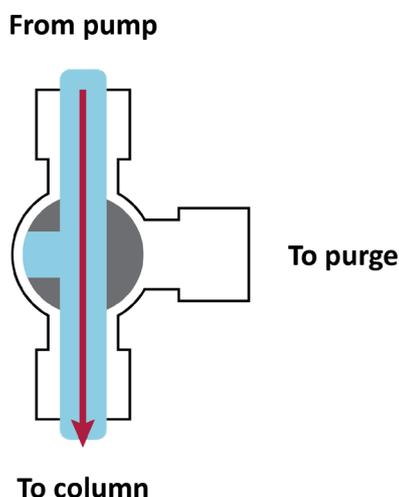
1. Connect one end of the three-way valve to the column inlet and the other end to the chromatography system pump. Leave the column outlet closed.
2. Configure the three-way valve flow path as shown in [Figure 6](#) below.
3. Attach a syringe to the purge line while pumping the mobile phase at low flow rate (i.e., 50 cm/hr). Draw the plunger to create negative pressure. Air bubbles will be drawn into the syringe, and mobile phase will immediately fill the space created.

Figure 6. Position of three-way valve for purging air from the column inlet



4. After all the air has been purged from the inlet line, engage the three-way valve as shown below in [Figure 7](#).

Figure 7. Position of three-way valve for flow to column



5. With the flow off and the three-way valve configured as shown in [Figure 7](#), open the column outlet and connect it to the chromatography system.
6. Introduce flow to the column at a low flow rate (i.e., 50 cm/hr) to flush trapped air from the column outlet.

In the absence of a three-way valve:

1. A “T” line can be connected between the column and chromatography system.
2. The “T” line can be used as described above for purging air.
3. After air has been purged, the purge line can be clamped or closed with a stopper.
4. The column outlet can then be connected to the chromatography system for normal use.

8.2 Appendix 2: Connecting an OPUS® Column to a chromatography system

1. Connect the inlet of the column to the chromatography system tubing set. At this point, the outlet of the column should be closed and disconnected.

Note: Use of stainless steel valves connected directly to the inlet and/or the outlet ports is not recommended. The additional weight to the top of the column will increase the risk of damage to the column hardware.

2. Start flowing the mobile phase through the system at <50% of the recommended operating flow rate for your chromatography resin/process. During this operation, the flow will be split. One portion of the mobile phase will enter the bypass line and the other portion will enter the inlet line.

Note: The mobile phase will not enter the column because the column outlet is closed (with the pinch valve or outlet cap) thus creating a stop barrier for the flow. The fraction of the flow that enters the inlet line will dislodge the trapped air in the tube and connector.

3. With the flow split to the bypass and inlet lines, air bubbles will begin to travel upward in the inlet tube and will be evacuated through the column bypass line into the system outlet. To ensure all the air is evacuated, tap and/or shake the inlet tube and inlet connector.
4. After all the air has been removed from the column inlet and connector, close the bypass line pinch valve and connect the outlet line to the column with the outlet pinch valve in the open position.
5. Run mobile phase through the column to purge the outlet of air.

8.3 Appendix 3: Column performance testing

Follow the steps below to measure the plate count and asymmetry of your OPUS® Column. Please note, minor differences (plus or minus 10-20%) in the measured plate count and asymmetry noted on the column CoA or CoQ are to be expected. Sources of variation include:

- Chromatography instruments for measurement
- Chromatography system
- Operator variability
- Normal variability within the test methods
 - Flow rate
 - Sample volumes
 - Equilibration/plug solutions
 - Injection method

If the plate count and asymmetry measurements are within defined acceptance limits (reference the column's CoA or CoQ QC Release Data), the column should be considered fit for purpose.

1. Remove column storage solution.
2. If column storage solution is alcohol based, run equilibration buffer at low flow rate (i.e., 50 cm/hr) for 2 - 3 column volumes (CVs). Ethanol solution is more viscous than water so the flow rate of this step should be chosen such that the pressure drop on the column does not exceed the maximum operating pressure.
3. After the storage solution has been removed, condition the column with the equilibration buffer for 1-2 column volumes at column qualification testing flow rate.
4. Proceed to testing the column:

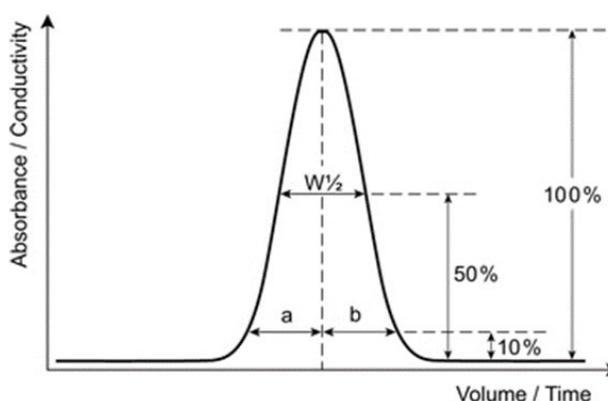
Note: Refer to your column's CoA or CoQ for the test "Mobile Phase" and "Testing Flow Rate" used by Repligen.

5. Conduct a pulse injection with 1-2% CV of the Injection Solution.

Note: Refer to your column's CoA or CoQ for the "Injection Solution" and "Injection Volume" used by Repligen.

6. Elute with mobile phase for 1-2 CV at the same test flow rate while monitoring UV or conductivity depending on the Injection Solution.
 - Salt injection solutions are typically analyzed with a conductivity meter while acetone injection solutions are analyzed with an UV meter.
 - Determine HETP (height equivalent to a theoretical plate) and A_s (peak asymmetry).

Figure 8. Absorbance/conductivity graph



Calculate HETP and A_s from the absorbance/conductivity curve (Figure 8) as follows:

$$HETP = \frac{L}{N}$$

L = Bed height (cm)

N = Number of theoretical plates (assuming a Gaussian peak)

$$N = 5.54 \times \left(\frac{V_e}{W_{1/2}} \right)^2$$

V_e = Peak elution distance

$W_{1/2}$ = Peak width at half peak height

Note: V_e and $W_{1/2}$ must be in the same unit.

Peak asymmetry (A_s) factor calculation:

$$A_s = \frac{b}{a}$$

a = 1st half peak width at 10% of peak height

b = 2nd half peak width at 10% of peak height

If the plate count and asymmetry measurements are within defined acceptance limits (reference the column's CoA or CoQ "QC Release Data") then the column should be considered fit for purpose.

For column comparison purposes, column efficiency is defined as the number of theoretical plates per meter (N/m). To calculate column efficiency, convert HETP from centimeters to plates/meter; see example below. A larger value for efficiency (N/m) indicates a sharper peak.

$$Efficiency = \frac{\text{Number of theoretical plates}}{\text{meter}} = \frac{N}{m} = \frac{100}{HETP}$$

Example:

Given a HETP specification of ≤ 0.05 cm/N, the conversion follows as below.

$$\frac{1}{0.05 \frac{cm}{N}} = 20 \frac{N}{cm}$$
$$20 \frac{N}{cm} * \frac{100 cm}{1 m} = 2000 \frac{N}{m}$$

Since the formula takes the reciprocal of ≤ 0.05 cm/N, the inequality inverts to greater than or equal to (\geq). Therefore, the converted efficiency specification is ≥ 2000 N/m.

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