

Connection and Priming

OPUS® Pre-packed Chromatography Columns

User Guide

OPUS®
30 cm



OPUS®
45 cm



OPUS®
60 cm



OPUS®
80 cm



OPUS®
10 cm



OPUS®
12.6 cm



OPUS®
14 cm



OPUS®
20 cm



OPUS®
25 cm



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1. Intended use

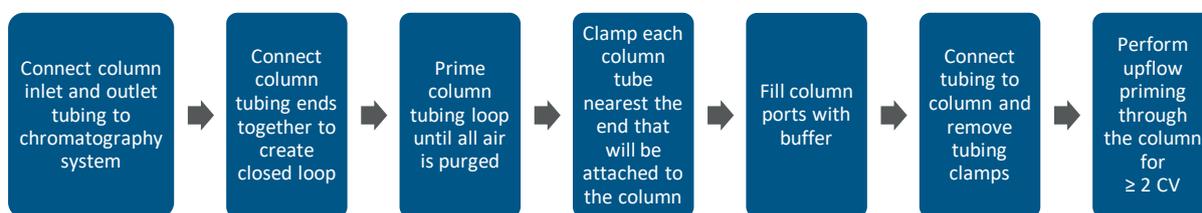
This document serves as a guide for connecting an OPUS® Pre-packed Chromatography Column to your chromatography system. The following recommendations serve as best-practices to help ensure an air-free column connection and optimal column performance.

Notes:

- 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.
- 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 as described in [Section 2](#).
- Tubing length of < 50 cm is suggested to minimize hold up volume of the system.

1.1 OPUS® Column connection and priming procedure (using flexible tubing)

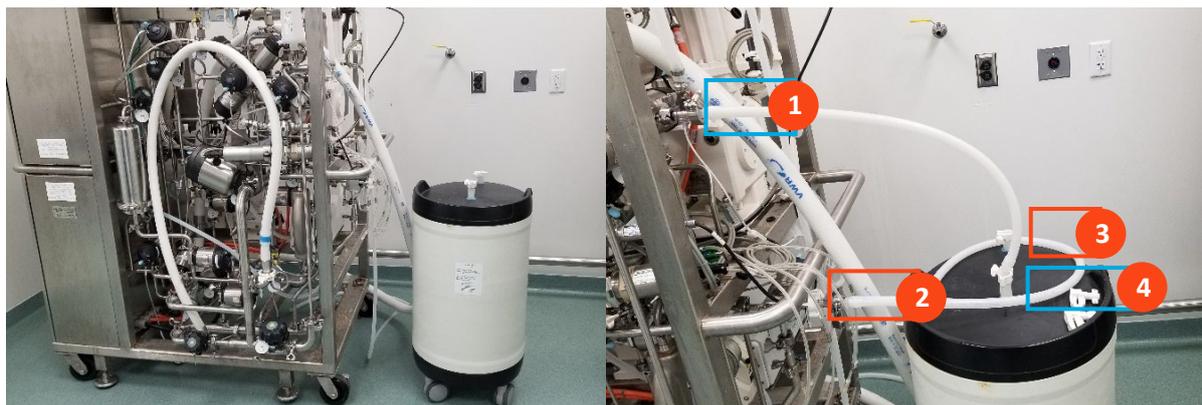
Figure 1. Procedural overview (flexible tubing)



1.2 Sizing of OPUS® Column inlet and outlet flexible tubing

Before you connect your OPUS® Pre-packed Chromatography Column to your chromatographic system, put the OPUS® Column in place to measure and procure appropriately sized tubing to connect the OPUS® Column to your chromatography system. When measuring the length of tubing required, try to minimize tubing hold up volume for best column performance. Avoid installing reducers, valves, and other fittings whenever possible. See [Figure 2](#) as a suggestion for proper setup.

Figure 2. Measure column tubing and connect to chromatography system

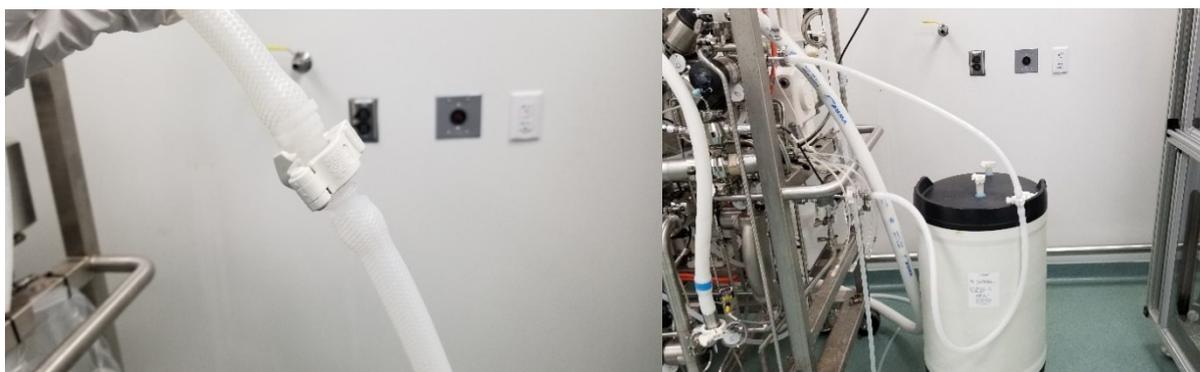


1. Skid column inlet
2. Skid column outlet
3. Column outlet
4. Column inlet

1.3 Priming of OPUS® Column flexible tubing loop

After acquiring the appropriate tubing lengths for connection to your OPUS® Pre-packed Chromatography Column, connect the tubing to both inlet and outlet of your chromatography system. Connect the free ends (the ends of the tubing not attached to the system) to one another, creating a closed loop, leaving the column disconnected as seen in [Figure 3](#). Once the column tubing is connected and the loop is closed, prime out the loop with sufficient volume of buffer to ensure the tubing is purged of all air. Ensure the priming flow occurs at a greater flowrate than the maximum production flowrate. Once the column tubing is fully primed, clamp each tube (as close to the end as possible) as seen in [Figure 4](#). Disconnect the inlet and outlet tubing from one another. Inspect the tubing to ensure each segment is still saturated with liquid and is not leaking at the tube clamp.

Figure 3. Connect flexible tubing without column in line and prime tubing loop



Column pictured above is NOT inline.

Figure 4. Clamp off flexible tubing after priming close to tubing connection



1.4 Filling OPUS® Column ports

Once the column tubing is primed and ready for connection to your OPUS® Pre-packed Chromatography Column, remove the caps from the inlet and outlet ports on your OPUS® Column. After opening the column ports, visually inspect the ports to determine if they are fully primed with buffer. If any air is observed in either column port, fill the port with buffer until the port is entirely filled with liquid. See [Figure 5](#) for depiction of filling the ports with buffer to reduce air introduction into the packed resin bed.

Figure 5. Inspect ports for air and fill with buffer



1.5 OPUS® Column Connection

Refer to [Figure 6](#) for the OPUS Column Connection Section.

After fully priming both your column tubing and column inlet and outlet ports, disconnect the column tubing ends from one another. Set your chromatography skid for operation with < 50 cm/hr linear velocity flow rate. Remove the pinch clamp from the outlet tubing with the tubing raised to maintain the equilibration buffer/water level within the tubing. Connect the outlet tubing to the outlet port of the column with a tri-clamp. Remove the pinch clamp from the inlet tubing with the tubing raised to maintain the equilibration buffer/water level within the tubing. Start liquid flow and connect column inlet tubing to the column inlet port with a tri-clamp.

During the connection of the inlet tubing to the inlet port, reduce air introduction into the line as a liquid-liquid connection is ideal.

Figure 6. Connect flexible tubing to column ports and then remove clamps

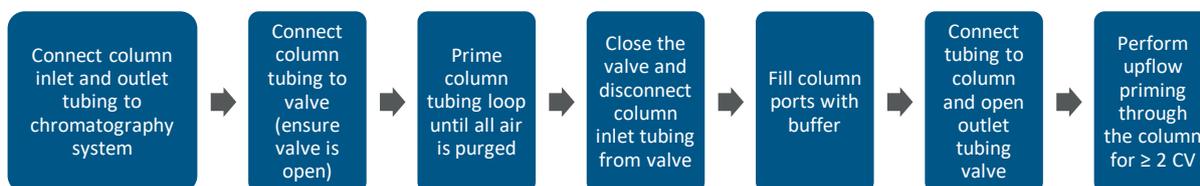


1.6 OPUS® Column priming

In the chance that some small amount of air is introduced to the OPUS® Pre-packed Chromatography Column during connection to the chromatography system, up-flow priming is recommended for all OPUS® Columns immediately following connection to your chromatography system. To ensure all remaining air is purged from the OPUS® Column, startup-flow through the column with at least 1 CV of buffer at 50 - 75% of intended operational flow velocity. Continue flushing the column while gradually increasing flow rate (~50 cm/hr every 2 minutes) for another 1 CV of buffer. Hold the flow rate steady for an additional 2 - 3 CV when 50 - 75% of the column packing pressure is achieved. **Do not exceed the OPUS® Column packing pressure at any point during priming and reduce pump flow rate as necessary.** Always follow the up-flow priming step with at least 2 CV of downflow at operational flow rate prior to column testing.

2. Large skid OPUS® Column connection and priming procedure (using rigid tubing)

Figure 7. Procedural overview (rigid tubing)



In this procedure an in-line 3-way valve is used to keep the column outlet tubing fully primed until connection with the column is established. The use of a valve is recommended only for large columns (> 0.5 L column) where the holdup volume of the valve will not be a significant portion of the column volume.

2.1 Sizing of OPUS® Column inlet and outlet rigid tubing

Before you connect your OPUS® Pre-packed Chromatography Column to your chromatographic system, put the OPUS® Column in place to measure and procure appropriately sized tubing to connect the OPUS® Column to your chromatography system. When measuring the length of tubing required, try to minimize tubing hold up volume for best column performance. Avoid installing reducers, valves, and other fittings whenever possible.

Figure 8. Column inlet and outlet rigid tubing



1. Skid column inlet
2. Skid column outlet
3. Column outlet
4. Column inlet

2.2 Priming of OPUS® Column rigid tubing loop

After acquiring the appropriate tubing lengths for connection to your OPUS® Pre-packed Chromatography Column, connect the tubing to both inlet and outlet of your chromatography system. Connect the free ends (the ends of the tubing not attached to the system) to a multi-way valve, creating a closed loop. Once both column tubes are connected to the valve (make sure the valve flow path is open) and the loop is completed without the column connected, prime out the loop with sufficient volume of buffer to ensure the tubing is purged of all air as shown in Figure 8.

Ensure the priming flow occurs at a greater flowrate than the maximum production flowrate. Once the column tubing is fully primed, stop flow, close the valve as shown in [Figure 9](#) and then disconnect the inlet tubing from the valve. Leave the outlet tubing connected to the valve to keep the outlet tubing fully primed. Inspect the outlet tubing to ensure it is still saturated with liquid and is not leaking out the valve.

Note: You can also use two valves (one on the end of both inlet and outlet tubing) to keep both column inlet and outlet tubing fully primed. Here one valve is used to minimize holdup volume in the tubing setup.

Figure 9. Connect rigid tubing without column in line using a valve and prime tubing loop

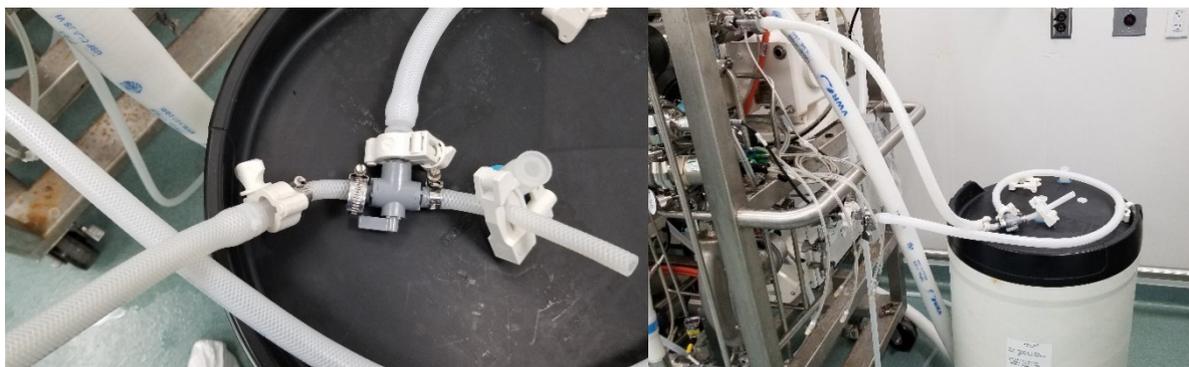


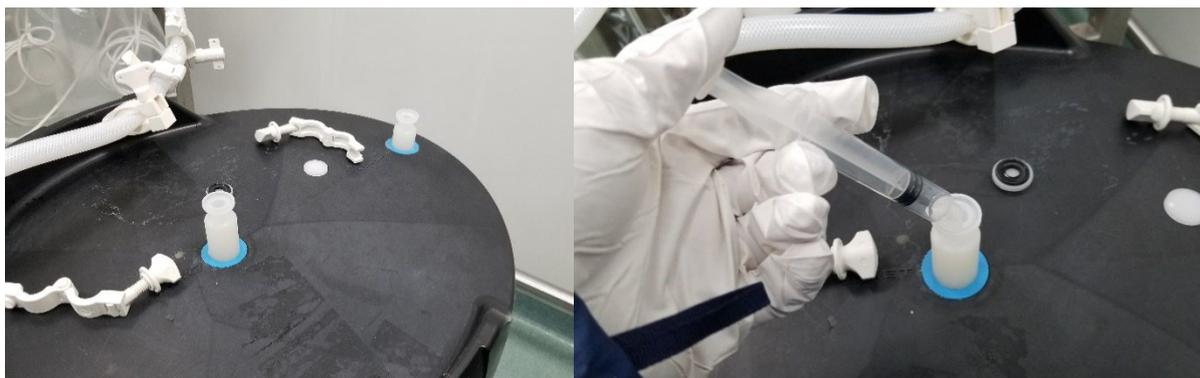
Figure 10. Close valve to block column outlet tubing after prime is complete



2.3 Filling OPUS® Column ports

Once the column tubing is primed and ready for connection to your OPUS® Pre-packed Chromatography Column, remove the caps from the inlet and outlet ports on your OPUS® Column. After opening the column ports, visually inspect the ports to determine if they are fully primed with buffer. If any air is observed in either column port, fill the port with buffer until the port is entirely filled with liquid.

Figure 11. Inspect ports for air and fill with buffer



2.4 OPUS® Column connection

After fully priming both your column tubing and column inlet and outlet ports, proceed with connecting your chromatography system to your OPUS® Pre-packed Chromatography Column. **For chromatography skid tubing that has a valve attached at the end, attach a short segment of tubing from the valve for direct connection to the OPUS® Column port.** If two valves are used, this will need to be executed for the assembly on each of the column connection ports. Refer to [Figure 12](#) for final valve assembly in relation to the port connection.

Figure 12. Connect valve to column outlet



Set your chromatography skid for operation with < 50 cm/hr linear velocity flow rate. Connect the outlet valve assembly to the outlet port of the column with a tri-clamp. Open the outlet valve assembly in preparation of flow from the column back to the chromatography skid as seen in [Figure 13](#). Open the inlet valve assembly with the tubing raised to maintain the equilibration buffer/water level within the tubing. Start liquid flow and connect column inlet valve to the column inlet port with a tri-clamp. **During the connection of the inlet tubing to the inlet port, reduce air introduction into the line as a liquid-liquid connection is ideal.**

Figure 13. Connect inlet tubing

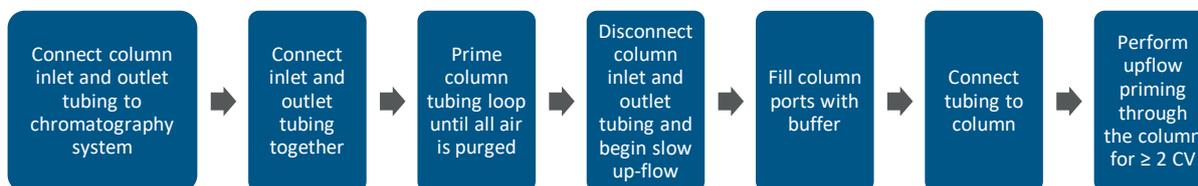
2.5 OPUS® Column priming

In the chance that some small amount of air is introduced to the OPUS® Pre-packed Chromatography Column during connection of the valve assemblies, up-flow priming is recommended for all OPUS® Columns immediately following connection. To ensure all remaining air is purged from the OPUS® Column, start up-flow through the column with at least 1 CV of equilibration buffer/water at 50 - 75% of intended operational flow velocity. Continue flushing the column while gradually increasing flow rate (~50 cm/hr every 2 minutes) for another 1 CV of flow. Hold the flow rate steady for an additional 2 - 3 CV when 50 - 75% of the column packing pressure is achieved. **Do not exceed the OPUS® Column packing pressure at any point during priming and reduce pump flow rate as necessary.** Always follow the up-flow priming step with at least 2 CV of downflow at operational flow rate prior to column testing.

3. Small skid OPUS® Column connection and priming procedure (using rigid tubing)

3.1 Procedural overview (small columns with rigid tubing)

Figure 14. Procedural overview (small columns with rigid tubing)



In this procedure the flow is maintained in the up-flow direction as the connection to the OPUS® Pre-packed Chromatography Column is made. This keeps the outlet tubing fully primed without the use of a clamp or valve.

3.2 Sizing of small OPUS® Column Inlet and outlet rigid tubing

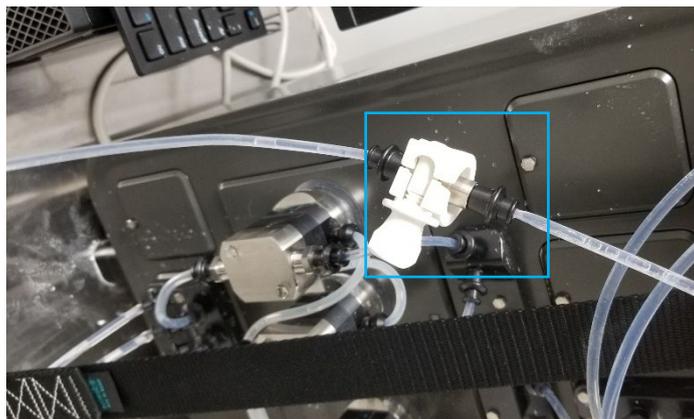
Before you connect your OPUS® Pre-packed Chromatography Column to your chromatographic system, put the OPUS® Column in place to measure and procure appropriately sized tubing to connect the OPUS® Column to your chromatography system. When measuring the length of tubing required, try to minimize tubing hold up volume for best column performance. Avoid installing reducers, valves, and other fittings whenever possible. See [Figure 15](#) for Tri-clamp connection assembly.

Figure 15. Small column inlet and outlet connectors



3.3 Priming of small OPUS® Column with rigid tubing loop

After acquiring the appropriate tubing lengths for connection to your OPUS® Pre-packed Chromatography Column, connect the tubing to both inlet and outlet of your chromatography system. Connect the free ends (the ends of the tubing not attached to the system) together, creating a closed loop, leaving the column disconnected ([Figure 16](#)). Once both tubing segments are connected and the loop is completed, prime out the loop with sufficient volume of buffer to ensure the tubing is purged of all air. Ensure the priming flow occurs at a greater flowrate than the maximum production flowrate. Once the column tubing is fully primed, stop flow, but leave the column tubing loop closed. Inspect the tubing to ensure it is still filled with liquid and is not leaking at any connection.

Figure 16. Connect small column tubing ends and prime rigid tubing loop

3.4 Filling small OPUS® Column ports

Once the column tubing is primed and ready for connection to your OPUS® Pre-packed Chromatography Column, remove the caps from the inlet and outlet ports on your OPUS® Column. After opening the column ports, visually inspect the ports to determine if they are fully primed with buffer. If any air is observed in either column port, fill the port with buffer until the port is entirely filled with liquid ([Figure 17](#)).

Figure 17. Inspect ports for air and fill with buffer

3.5 Small OPUS® Column connection

After fully priming both your column tubing and column inlet and outlet ports, disconnect the column inlet and outlet tubing from one another ([Figure 18](#)). With the tubing raised to maintain the equilibration buffer/water level within the tubing, start buffer flow from the tubing designated for attachment to the outlet port with low flow rate (50 cm/hr is recommended to minimize overflow of buffer from outlet tubing). As buffer flow is maintained, connect column outlet tubing to the column outlet and then column inlet tubing to the column inlet ([Figure 19](#)). **During the connection of the inlet tubing to the inlet port, reduce air introduction into the line as a liquid-liquid connection is ideal.**

Figure 18. Disconnect small column tubing ends for connection to outlet port



Figure 19. Connect rigid tubing to small column outlet and inlet



3.6 Small OPUS® Column priming

In the chance that some small amount of air is introduced to the OPUS® Pre-packed Chromatography Column during connection of the valve assemblies, up-flow priming is recommended for all OPUS® Columns immediately following connection. To ensure all remaining air is purged from the OPUS® Column, start up-flow through the column with at least 1 CV of equilibration buffer/water at 50 - 75% of intended operational flow velocity. Continue flushing the column while gradually increasing flow rate (~50 cm/hr every 2 minutes) for another 1 CV of flow. Hold the flow rate steady for an additional 2 - 3 CV when 50 - 75% of the column packing pressure is achieved. **Do not exceed the OPUS® Column packing pressure at any point during priming and reduce pump flow rate as necessary.** Always follow the up-flow priming step with at least 2 CV of downflow at operational flow rate prior to column testing.

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