User Guide

OPUS® Pre-packed Columns

1.2 cm - 8 cm Internal Diameter

REPLIGEN

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Safety Notices:

- Unless otherwise specified by the end-user, the column is generally shipped with resin in 18.5% ±1% ethanol solution, a recognized bacteriostatic agent. It is flushed from the resin during equilibration and preparation for use. Please refer the applicable OPUS® Work Order for confirmation of the storage solution.
- Follow all local regulations for safe disposal
- For laboratory and manufacturing production use only
- Not for administration to humans

Contents

1.0. Introduction

What is Open Platform User Specified?

OPUS® Open Platform User Specified 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.

Column Design:

OPUS® 1.2 to 8 cm ID columns are designed for process development and bench scale applications. The columns are manufactured with class VI, medical grade materials, including Nylon 6-6, polypropylene (PP), and polyethylene (PE) and are available in a range of diameters and bed heights. These columns are compatible with any HPLC, FPLC™ or AKTA™ system.

Table 1: Column Physical Characteristics

Size	Components	Connections	Max. Back Pressure[1]
1.2 cm IDs	Column Body: Polypropylene Unit ends: Type 6-6 Nylon 101 with 10µm Titanium Frit	¼-28 Threaded Flat Bottom Ports with 0.055" diameter through hole ¼-28 Flangeless Ferrule and Nut for connection to 1/16" OD tubing included	5 Bar
2.5 cm IDs	Column Body: Polyethylene (MDPE) Unit ends: Type 6-6 Nylon 101 with 300µm Nylon mesh and 5µm Nylon mesh	¼-28 Threaded Flat Bottom Ports with 0.063" diameter through hole ¼-28 Flangeless Ferrule and Nut for connection to 1/16" OD tubing included	5 Bar
5 cm IDs	Column Body: Polypropylene (PP) Unit ends: Polypropylene with 12µm polypropylene mesh	¾ inch Mini TC connections with 0.125" diameter through hole	5 bar
8 cm IDs	Column Body: Type 6-6 Nylon 101 Unit ends: Type 6-6 Nylon 101 with 300µm Nylon mesh and 5µm Nylon mesh	¾ inch Mini TC connections with 0.18" diameter through hole	5 bar

- Working Temperature range is 4 – 30°C
- Typical storage solution 2/3 PBS, 18.5% EtOH. Please refer the applicable OPUS® Work Order for confirmation of the storage solution.
- Chemical compatibility with most aqueous chromatographic buffers and solvents used for purification of biologics.

[1] Most agarose resins should be operated below 4 bar. Prior to using the column, consult the resin manufacturer's instructions for the maximum pressure which the resin can withstand.

Table 2: Conversion from linear to volumetric flow rates:

Column Diameter	Flow at 100 cm/h	Flow at 200 cm/h	Flow at 300 cm/h
1.2 cm	1.9 mL/min	3.8 mL/min	5.7 mL/min
2.5 cm	8.2 mL/min	16.4 mL/min	24.6 mL/min
5 cm	32.7 mL/min	65.4 mL/min	98.2 mL/min
8 cm	83.8 mL/min	167.6 mL/min	251.4 mL/min

2.0. Operation

2.1. Chromatography System Set Up

System Pressure

Note: It is recommended to set the system pressure alarm at 4 bar (60 psi, 0.4 MPa) to provide an appropriate safety factor. Failure to set a pressure alarm could result in pressures that could compromise the media and/or column structure.

Typical operating pressures for columns packed with agarose based resins are usually < 4 bar. Repligen recommends a back-pressure regulator be used with all systems to prevent degassing in the detector flow cell.

All buffers or feedstock solutions applied to the column should be filtered with 0.22 µm filter. Repligen also recommends using a 2 µm in-line filter upstream of the column.

2.1.1. Column System Connection: 1.2 and 2.5 cm Internal Diameter Columns

OPUS® 1.2 cm and 2.5 cm ID columns have identical, ¼-28 threaded flat bottom inlet and outlet ports.

1. After opening the box, follow these instructions:
   - Inspect the outside of the cardboard carton for any unusual signs of damage. If significant damage has occurred, please contact Repligen immediately.
   - Locate the shipping delivery documents attached to the outside of the box
   - Locate the Certificate of Quality (COQ) for the column inside the box.
   - Remove the protective foam to expose the column.

2. Remove the column from the box using the following instructions:
   - For maximum cleanliness, Repligen recommends keeping the clear plastic bag containing the column intact for Step 2.
   - Use two hands to grab the column, and lift it from the box.

3. If you are not ready to use the column, leave the clear plastic bag intact and store the column according to the recommendations of the resin manufacturer.

4. When you are ready to use your OPUS® column, remove the clear plastic bag containing the column.

For ¼-28 Threaded Connections:

1. Remove the upper stopper from the column and attach a suitable ¼-28 threaded fitting to the OPUS® column. The connection should be made "drop-to-drop" to avoid introduction of air into the system. Turn the ¼-28 threaded fitting connector nut clockwise until it is finger-tight. Do not over-tighten the fitting.

2. Remove the bottom stopper from the OPUS® column, and repeat the connection process described above, connecting the ¼ -28 threaded outlet to the OPUS® column and to the chromatography system.

3. Before startup, please consult the resin manufacturer's instructions for recommended conditioning and equilibration procedures. In general, OPUS® columns should be flushed with at least 4-6 column volumes of RO/DI water or mild buffer (e.g. PBS) at 100 cm/h. Equilibrate the column by following the initial column flush with a second flush of 4 – 6 column volumes of the desired equilibration buffer.

For 1/16" Tubing Connections:

WARNING: When used directly with a 1/16" tubing connection (e.g. an AKTA System connectors) the ¼-28 OPUS® column threads may leak.

To avoid leaks when using a 1/16" tubing connection each 1.2 cm and 2.5 cm internal diameter OPUS® column should be used with ¼-28 flangeless ferrule fittings. Repligen recommends Upchurch Scientific® ¼-28 flangeless ferrule fittings for connecting to 1/16" OD tubing (catalog numbers P-200x and P-210N). Instructions on how to install Upchurch Scientific connections can be found in Appendix 1.

- Before startup, please consult the resin manufacturer's instructions for recommended conditioning and equilibration procedures. In general, OPUS® columns should be flushed with at least 5 column volumes of RO/DI water or mild buffer (e.g. PBS) at 100 cm/h. Equilibrate the column by following the initial column flush with a second flush of 2 – 5 column volumes of the desired equilibration buffer.

2.1.2. Column System Connection: 5 and 8 cm Internal Diameter Columns

1. After opening the box follow these instructions:
   - Inspect the outside of the cardboard carton for any unusual signs of damage. If significant damage has occurred, please contact Repligen immediately.
   - Locate the shipping delivery documents attached to the outside of the box.
   - Locate the Certificate of Analysis (8 cm IDs) or Certificate of Quality (5 cm IDs) for the column inside the box.

2. If you are not ready to use the column, leave the clear plastic bag intact and store the column according to the recommendations of the resin manufacturer.

3. When you are ready to use your OPUS® column, remove the clear plastic bag containing the column.
   a. For 5 and 8 cm ID columns, the inlet and outlet will be sealed with SaniSure CLAMPs (see www.sanisure.com for more information). Using a wire cutter or sharp scissors, remove the white cable-tie on the SaniSure® CLAMPs* on both the inlet and the outlet.

4. With the inlet and the outlet open, hook up the column inlet under low flow so that no air is introduced into the column (a 3-way valve can also be used for this connection). Once the inlet has been connected under low flow, the outlet can then be connected.
   - Please reference Table 1 of this user guide for the inlet and outlet connection port size of your column.
   - Instructions for using a 3-way valve to purge air from the inlet line can be found in the Appendix 2.

5. Flush the storage solution (example: 18-20% EtOH) from the column with at least 4-6 column volumes of RO/DI water or mild buffer (e.g. PBS) at 100 cm/h.
   - Note: All the buffers and cleaning solutions should be filtered through a 0.22 µm filter membrane.

6. To test for chromatographic performance, a short instruction guide can be found in Appendix 3.

7. Equilibrate your column using at least 4-6 CVs of your beginning process buffer.

8. Reference the technical specifications below when using your OPUS® column during processing:
   - **Cleaning and Storage**: OPUS® columns do not require special in-process cleaning or storage protocols. Please consult the resin manufacturer for cleaning and storage information.
   - **Mechanical Strength**: Recommended maximum 5 bar working pressure.
   - **Temperature**: Column construction is designed to support a working temperature range of 2°C to 40°C.

9. 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 manufacturer or other validated procedure.

10. Reuse post storage: Start with the general usage instructions in Step 5.

3.0. Troubleshooting

3.1. Air in the column

Potential Fixes

- If air entered the inlet port only and did not reached the column (to the best assessment of the operator), follow the air purge procedure described in Appendix 2.

- If air entered the packed chromatography bed, recondition the column by running a solution with lower surface tension (e.g. 20% ethanol for normal phase resin, or 60% ethanol for reverse phase resins, or 1% surfactant: check compatibility with resin manufacturer) in reverse flow, at 20% of operating flow rate for a minimum of 5 hours.

- Test column performance (HETP, asymmetry) according to instructions in Appendix 3.

3.2. Pressure increase during run

Causes

- Obscuration of the bed support or inlet frit
- Incorrect column valve position
- Operation under higher flow rate than recommended for the packed resin
- Residue build up at the head of the column
- Compromised chromatography resin

Potential Fixes

- Run the column in reverse at normal operating flow rate for the specific resin for at least 10 CV. Recheck pressure and column performance (HETP, asymmetry) under normal operating conditions.
- Clean the column with the appropriate cleaning method, preferably in reverse flow, for the residue that clogged the mesh and/or resin.
- Check valve position.

3.3. Pressure drop during run

Cause

- Line or fitting leaks

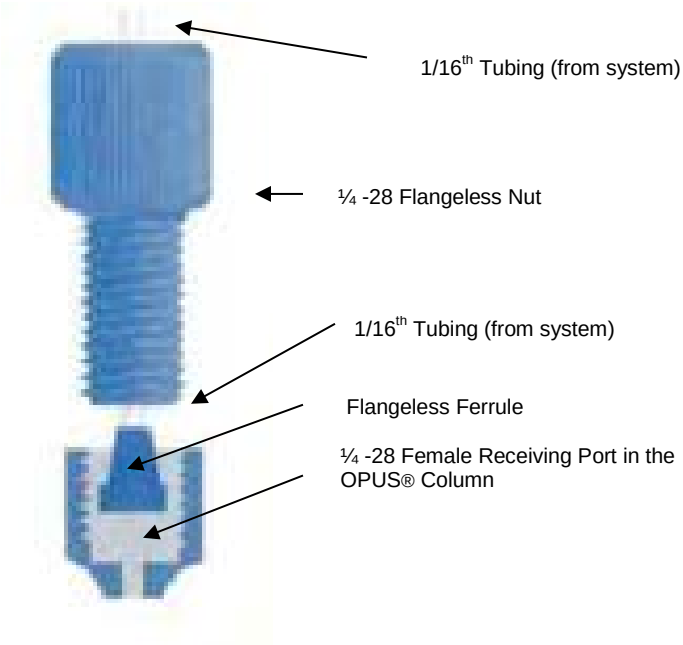
Potential Fixes

- Check lines and connections.

4.0. Appendices

4.1. Appendix 1: Instructions for Installing Upchurch Scientific® 1/16" OD Tubing Connectors

Figure 1. Upchurch Scientific® 1/16" OD Tubing Connector



To minimize the risk of leaks resulting from improper connections, please follow these directions when connecting the 1/16th system tubing into the threaded flat-bottom receiving port of the column.[1]

1. For polymer tubing, cut the end of the tubing, leaving a square-cut face. If other tubing materials are being used (e.g. steel or other metallic tubing) ensure the end of the tubing is flat and burr-free.

2. Slide the flangeless nut over the tubing, with the nut threads facing the tubing end being connected.

3. Slip the flangless ferrule over the tubing, with the tapered portion of the ferrule facing towards the nut. Align the end of the tubing such that it just projects through the ferrule, and slide the tubing and ferrule back so it touches the bottom of the flangeless nut.

4. Insert the flangeless nut, tubing, ferrule assembly in place into the ¼-28 female receiving port of the OPUS® column, and while holding the tubing down firmly into the port, tighten the nut finger-tight. The action of tightening the flangeless nut seals the ferrule to both the tubing and the nut.

[1] Instructions For Use, Flangeless Fittings, IDEX Health and Sciences LLC – Home of Upchurch Scientific Products, P01 11/09

5. Once installation of the Upchurch Scientific© connectors is complete, please consult the resin manufacturer's instructions for recommended conditioning and equilibration procedures. In general, OPUS® columns should be flushed with at least 4-6 column volumes of RO/DI water or mild buffer (e.g.

   PBS) at 100 cm/h. Equilibrate the column by following the initial column flush with a second flush of 2 – 5 column volumes of the desired equilibration buffer.

Note: If you experience difficulty connecting the column to your system, please contact Technical Service at +1-781-250-0111 (option 3) or technical.support@repligen.com for further assistance.

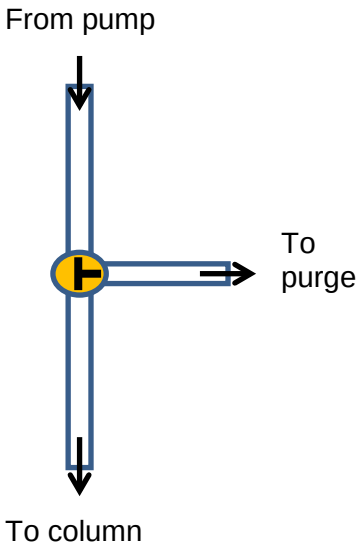
4.2. Appendix 2: Use of a 3-Way Valve for Air Purging

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

1. Connect one end of the 3-way valve to the column inlet and the other end to the chromatography system pump. **LEAVE THE COLUMN OUTLET CLOSED**.

2. Configure the 3-way valve flow path as shown in Figure 2 below.

Figure 2. 3-way valve position for purging air from the column inlet

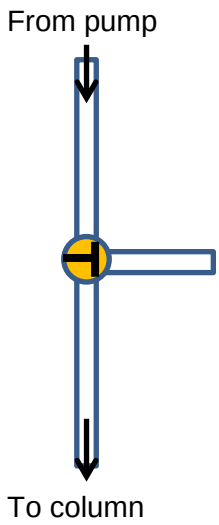


3. Attach a syringe to the purge line, while pumping the mobile phase at low flow rate (half the normal operating flow rate), and draw the plunger to create negative pressure. Air bubbles will be drawn into the syringe, and mobile phase will immediately fill the space created.

4. To ensure all the air has been purged, tap the inlet line, or shake the column slightly. This process will dislodge any trapped air remaining in the line.

5. After all the air has been purged from the inlet line, engage the three way valve as shown in Figure 3 below.

Figure 3. 3-way valve position for column operation after air has been purged from the inlet line



6. With the flow off and the 3-way valve configured as shown in Figure 3, **open the column outlet** and connect it to the chromatography system.
7. Introduce flow to the column at a low flow rate (e.g. half the operating flow rate) to flush trapped air from the column outlet.

In the absence of a 3-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.

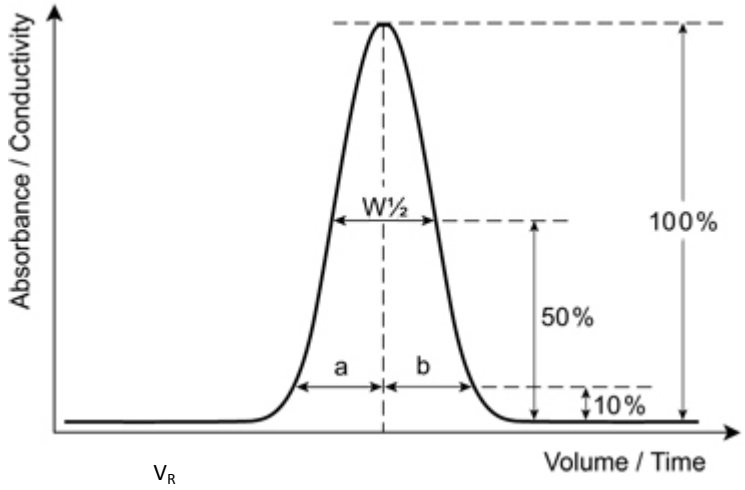
4.3. Appendix 3: Performance Testing (HETP / Asymmetry): Recommended equilibration and conditioning of the column for Performance re-testing

If column performance needs to be re-tested, the following steps should be followed:

- Remove column storage solution:
  - If column storage solution is 18-20% Ethanol, run RO/DI water at low flow rate for at least 5 column volumes. Because ethanol solution is more viscous than water, the flow rate of this step should be chosen such that the pressure drop on the column does not exceed the maximum operating pressure for the chromatography resin and is below 4 bar (the maximum tolerated pressure for the OPUS® column
  - If column storage solution is 0.1*M* NaOH run a higher ionic solution such as 0.2*M* NaCl or PBS at normal operating flow rate for at least 5 column volumes
- After the storage solution has been removed, condition the column with the packing buffer (consult OPUS® column work order for at least 5 column volumes at packing flow rate (typically 1.5 – 2 x the normal operating flow rate; consult packing instructions for the media)

- Proceed to testing the column:
  - Equilibrate with more than 4-6 column volumes of testing mobile phase at a low flow rate (as specified in the testing procedure on OPUS® Work Order form)
  - Conduct a pulse injection of 1 – 2 % CV of the test solution
  - Elute with elution buffer (equilibration buffer) for 3 – 4 CV at the same flow rate as equilibration flow rate, while monitoring the UV or conductivity

- Calculate number of theoretical plates and asymmetry of the eluted peak:



Theoretical Plate Count: $N = 5.54 \times (V_R/W_{1/2})^2$, assuming a Gaussian peak

Where:
N = number of theoretical plates
V_R = peak retention (elution) volume
$W_{1/2}$ = peak width at half height

Asymmetry: As = b/a

Where:
a = partial peak width at 10% of the peak height for the leading part of the peak
b = partial peak width at 10% of the peak height for the tailing part of the peak

- If after following the above steps discrepancies still exist between the Certificate of Quality and re-test, they could be attributed to using different instruments for measurement, operator error, or normal variability within the test results. Please consult Repligen technical support +1-781-250-0111 (option 3) or technical.support@repligen.com for further questions.

- If re-test results are within acceptance limits, the column should be considered good to use.

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