# Technical
## User Guide

### SNAP® Laboratory Glass Columns

Next generation technology for high-performance preparative chromatography

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PURITY by DESIGN

# INTRODUCTION

**Developed with real world experience and customer feedback at the forefront of the design, the SNAP® laboratory column range provides effective, efficient, and user-friendly hardware solutions for liquid preparative chromatography. With extensive configurable options available, these glass columns are applicable to a range of bioprocessing applications such as process development and viral clearance studies. The columns can be implemented into almost any process, exhibiting durability, and delivering high performance. SNAP® columns can be configured with USP VI/FDA conforming material, with ADI-free statements also available. Please see the Validation Support Document for further information.**

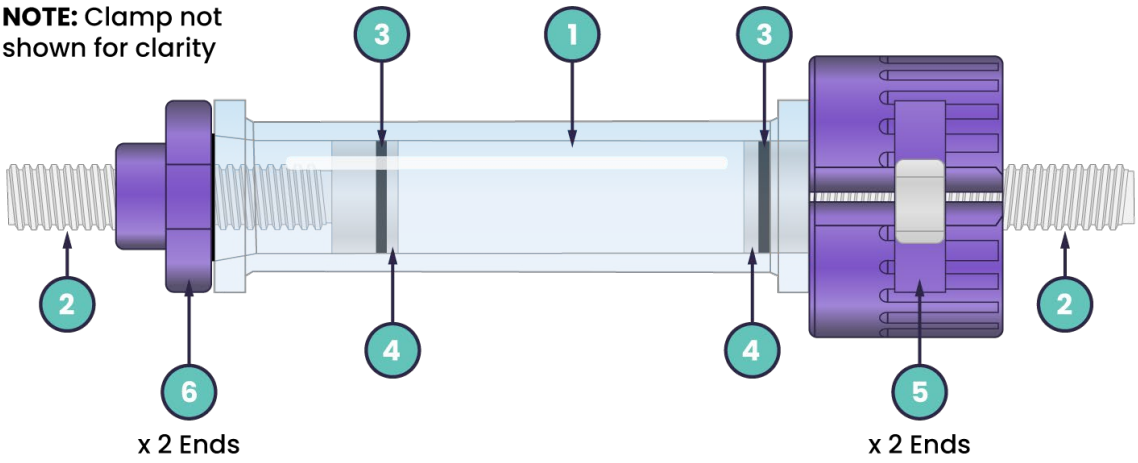
Careful choice in materials of construction, combined with customer feedback, has driven the design such that biocompatibility can be achieved in virtually any circumstance.

The patented SNAP® closure mechanism provides a safe and reliable means to access the column contents.

## Description of Components

1. Glass body
2. Pistons
3. O-Rings
4. Frits
5. Clamp Assembly
6. Piston Adjustment Nut

Each column is provided with two piston adjustment nuts and two clamp assemblies.



# SNAP® COLUMN PROPERTIES

SNAP® columns are available in different materials depending on the intended application. Biocompatibility should be verified with material selection of column configuration.

| | Aqueous Buffer (AB) version | Solvent Resistant (SR) version | Process-ready (PR) version |
|---|---|---|---|
| Temperature Range: | 4-40 ˚C | 4-80 ˚C | 4-40 ˚C |
| Frit | Polyethylene (PE) | Stainless Steel | Polyethylene (PE) |
| Sealing | Viton® | Kalrez® (or equivalent) | Viton® Or Kalrez® (or equivalent) |
| Piston | Acetal | PEEK | PEEK |
| Height Adjustment options | Short/Short, Short/Long, Long/Long pistons | Short/Short, Short/Long, Long/Long pistons | Short/Short, Short/Long, Long/Long pistons |
| Inlet /Outlet Connections | 1/4“-28 female screw thread | 1/4“-28 female screw thread | 1/4“-28 female screw thread supplied with TC adapter |

PEEK - polyether ether ketone

Aqueous buffers include commonly used reagents in chromatography processes up to working strengths.

Solvent resistant applications include the use of solvents such as acetonitrile, or where concentrations of commonly used solutions exceed those suitable for use with the aqueous configuration.

Process Ready columns use materials with additional certification to confer compliance to USP VI/FDA conforming material, with ADI-free statements also available

## Maximum Pressure Rating

SNAP® columns are designed to withstand increased pressure often associated with the use of smaller bead, higher performance chromatography media. The redrawn precision glass body meets the US and European Pharmacopoeia specifications for Type 1 borosilicate glass. Type 1 Class A 3.3 Expansion Borosilicate glass also conforms to Federal Specification DD-G- 5416 and ASTM E-438.

| Diameter (ID) | Aqueous Buffer (AB) and Solvent Resistant (SR) version | | Process-ready (PR) version | |
|---|---|---|---|---|
| | Pressure (BAR) | Pressure (PSIG) | Pressure (BAR) | Pressure (PSIG) |
| 10 mm | 40 BAR | 580 PSIG | 13 BAR | 188 PSIG |
| 15 mm | 35 BAR | 508 PSIG | 13 BAR | 188 PSIG |
| 25 mm | 24 BAR | 348 PSIG | 13 BAR | 188 PSIG |
| 35 mm | 18 BAR | 261 PSIG | 13 BAR | 188 PSIG |
| 50 mm | 13 BAR | 188 PSIG | 13 BAR | 188 PSIG |

# COLUMN INSTALLATION & OPERATION

Please read the operating instructions for this equipment prior to use. Do not exceed the recommended pressure limits stated on the label.

Carefully remove the SNAP® column from the packaging. Inspect for any missing components, damage, or defects before use.

- Prior to the first use of the column, clean all components with laboratory detergent and rinse thoroughly with distilled water.
- O-rings are shipped loose and should be installed prior to first use. O-ring material will vary with order specifications. If O-rings have been stored for any length of time they should be inspected prior to use for dryness and cracking.
- It is recommended to use degassed and pre-filtered solutions, as particulates may block the frits.
- Make sure that the porosity of the frits is appropriate for the media being used. We recommend that the frit porosity be at least 50% smaller than the average particle diameter.
- Columns should be sealed when not in use to avoid bed degradation and drying out of media.
- Prior to sample injection, ensure that no dead volume is present at the column inlet during the conditioning phase.
- If the desired packed bed height is greater than half of the maximum height of the column, it is recommended to use a packing adaptor. These are available to purchase separately.

# Packing the Column.

1. If required to obtain a fixed bed height, it is recommended to determine the slurry percentage of the media being packed. Refer to information from the media manufacturer for further guidance on this, as well as compression factors and recommended packing conditions.

2. Assemble the column by inserting the bottom piston into the 0 mm end of the glass column body. When inserting the piston, take care to insert straight into the glass body. Rotate the nut block until it contacts the glass and secure the clamp. Adjust as required by turning the clamp to move the piston up or down. For packing purposes, install the bottom piston with Frit fitted and clamp only.

3. Mount the column assembly vertically onto a suitable bracket for packing. Column stands are available for purchase separately. Attach appropriate tubing to the outlet of the column, and cap. Attach appropriate tubing to the unattached piston, and connect to the chromatography system to be used for packing.

4. Carefully pour the pre-prepared media slurry into the column in a single continuous step. Pouring the adsorbent down the side of the column helps to prevent air becoming trapped within the adsorbent bed. Ensure the complete transfer of the media into the column.

5. Allow the media to settle in the column until a dead volume of packing solution above the media bed of approximately 1 cm has formed.

6. Insert the top piston into the column so that it is submerged in the headspace formed above the media bed. Lower the piston adjustment nut so that it contacts the glass, attach the clamp, and fasten SNAP® latch. Remove cap from outlet tubing and direct it to waste.

7. Apply flow at the required flow rate and pressure for the media being packed. When the resin bed has compressed (typically after 2-5 column volumes), stop the flow. Disconnect the inlet tubing from the chromatography system and direct to waste, and disconnect the outlet tubing from the system and cap.

8. Slowly turn the top clamp counterclockwise; as the piston submerges into the headspace formed above the packed bed, excess buffer will flow up the piston into the waste. Stop lowering the piston just above the resin bed. Reconnect the inlet tubing to the chromatography system, uncap the outlet tubing and direct to waste.

9. Recommence flow, and once the bed compresses fully, stop the flow. Disconnect and cap the outlet tubing and unscrew the inlet tubing sending it to waste. Turn the clamp counterclockwise until the frit contacts the surface of the bed.

10. Reconnect the inlet and outlet tubing to the chromatography system. The column is ready to use.

# Packing the column using the SNAP® packing adaptor

It is recommended to use the SNAP® packing adaptor when the desired bed height is greater than half of the maximum bed height of the column. Packing adaptors are available for purchase separately. (If using a Process-ready SNAP® column ensure that a Process-ready SNAP® adaptor is purchased).

SNAP® column packing adaptors are easy to install and are the same diameter as the column they are mounted on, meaning issues with turbulent flow at the interface/joining point are avoided.

| SNAP® Columns (AB, SR) | | | Process-ready SNAP® (P) | |
|---|---|---|---|---|
| Column ID (mm) | Pressure Rating (bar) | | Column ID (mm) | Pressure Rating (bar) |
| 10 | 20 | | 10 | 13 |
| 15 | 17 | | 15 | 13 |
| 25 | 13 | | 25 | 13 |
| 35 | 9 | | 35 | 9 |
| 50 | 6 | | 50 | 6 |

**NOTE Pressure rating differs from whole column pressure rating**

Packing adaptors consist of a coupling unit and glass body of same column ID as the column to be packed. The O-ring material varies dependant on column configuration.

1. Allow all materials to equilibrate to the temperature at which the chromatography process is to be performed.

2. If required to obtain a fixed bed height, it is recommended to determine the slurry percentage of the media being packed. Refer to information from the media manufacturer for further guidance on this, as well as compression factors and recommended packing conditions.

3. Assemble the column by inserting the bottom piston into the 0 mm end of the glass column body. When inserting the piston, take care to insert straight into the glass body. Rotate the nut block until it contacts the glass and secure the clamp. Adjust as required by turning the clamp to move the piston up or down. For packing purposes, install the bottom piston with Frit fitted and clamp only.

4. Attach the packing adaptor on top of the column by opening the jaw and loosening the compression nut. Clip the split ring over the end of the glass column, fasten the latch, and fully tighten the compression nut; see diagram below.


5. Mount the column and packing adaptor assembly vertically onto a suitable bracket for packing. Attach appropriate tubing to the outlet of the column.

   **NOTE:** It is important to wet out the bottom frit before commencing column packing. To do this, add a small volume of packing buffer to the column assembly, and slowly draw through the outlet tubing with a syringe. This will encourage flow through the bottom frit. Attach a syringe or cap to the outlet tubing to seal.

6. Attach appropriate tubing to the unattached piston and connect to the chromatography system to be used for packing.

7. Carefully pour the pre-prepared media slurry into the column in a single continuous step. Pouring the adsorbent down the side of the column helps to prevent air becoming trapped within the adsorbent bed. Ensure the complete transfer of the media into the column.

8. Allow the media to settle in the column until a dead volume of packing solution above the media bed of approximately 1 cm has formed.

9. Insert the top piston into the packing adaptor so that it is submerged in the headspace formed above the media bed. Lower the piston adjustment nut so that it contacts the glass, attach the clamp, and fasten SNAP® latch. Remove syringe/cap from outlet tubing and direct it to waste.

10. Apply flow at the required flow rate and pressure for the media being packed. When the resin bed has compressed (typically after 2-5 column volumes), stop the flow. Disconnect the inlet tubing from the chromatography system and direct to waste, and disconnect the outlet tubing from the system and cap.

11. If the resin bed has compressed to be in the column only and not the packing adaptor, remove the top piston from the packing adaptor and pipette the excess solution out. The packing adaptor can then be removed from the column by opening the clamp. Reinsert the piston which was removed from the packing adaptor into the top of the column and secure.

12. Slowly turn the top clamp counterclockwise; as the piston submerges into the headspace formed above the packed bed, excess buffer will flow up the piston into the waste. Stop lowering the piston just above the resin bed. Reconnect the inlet tubing to the chromatography system, uncap the outlet tubing and direct to waste.

13. Recommence flow, and once the bed compresses fully, stop the flow. Disconnect and cap the outlet tubing and unscrew the inlet tubing sending it to waste. Turn the clamp counterclockwise until the frit contacts the surface of the bed.

14. Reconnect the inlet and outlet tubing to the chromatography system. The column is ready to use.

# COLUMN EFFICIENCY TEST

It is recommended to perform column efficiency testing on packed columns to assess the quality of the packed bed. The testing involves injecting a small sample of non-binding substrate onto the column at a set flow rate, and the detected elution peak assessed for asymmetry factor and plate count (HETP; height equivalent of theoretical plates).

Refer to the individual media manufacturer for recommended test conditions and specifications. Outlined below is a generic test method.

1. It is recommended to use a low to medium strength conductivity solution (e.g., 0.1 M NaCl) but will be dependent on the packed media.

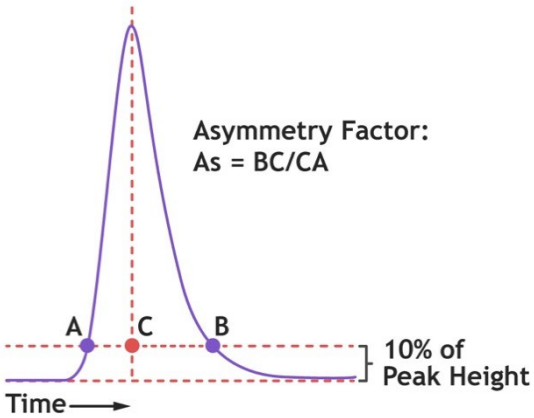
2. Attach the column to an equilibrated workstation.

3. Commence flow to equilibrate and obtain a baseline.

4. Inject a 2% to 5% CV of a non-binding tracer solution. This will depend on the media; examples include 1% to 2% acetone or a 1 M to 2 M NaCl solution.

5. Continue the flow until a UV or conductivity peak, depending on tracer solution used, is observed and the trace has returned to baseline.

6. End run and determine the asymmetry factor:




To calculate the theoretical plate count at half peak height, assuming a Gaussian peak:

$$N = 5.54 \times (Ve / W_{½})^2$$

Where:
- N = number of theoretical plates
- Ve = peak max (elution) volume (C) from tracer solution injection
- $W_{½}$ = peak width at half height

# CONNECTING THE COLUMN

Ensure appropriate connections are made to any chromatography system when connecting the column and avoid cross threading. Securely fasten the inlet and outlet connections to ensure that leakage does not occur during use.

On all SNAP columns the connection on the end of the pistons is a standard 1/4"-28 HPLC flat bottom

For Aqueous Buffer and Solvent resistance versions connectors are provided for either 1/16" or 1/8" tubing, depending on column diameter.

For Process ready revision a TC adaptor with O ring is fitted to the column. These connector and O-ring are composed of compliant materials and the certificate is supplier in the validation support document.

When connecting a chromatography column to a system the internal diameter of the tubing should be consistent with the column inlet diameter and the system. Introduction of valves, larger bore tubing, large sensor flow cell will influence the chromatography performance.

# FRIT REPLACEMENT

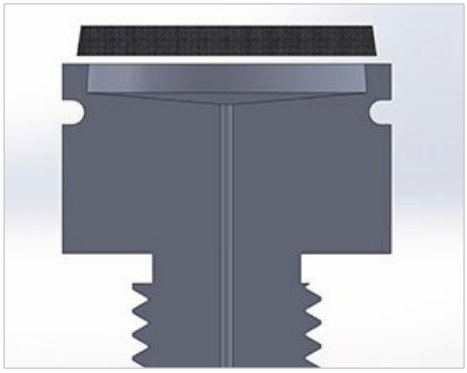
Each piston is supplied with a porous frit. Material and frit porosity will vary with order specifications. Polyethylene frits can be removed for replacement and cleaning.

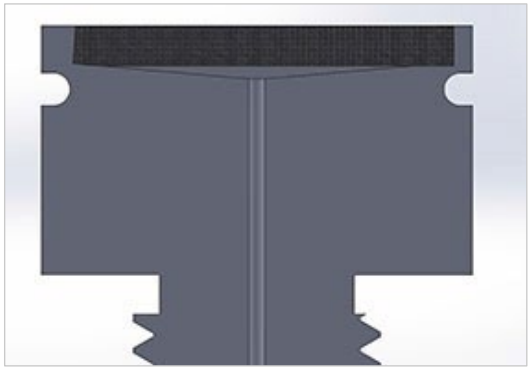
To remove the frit, insert the frit ejector tool into the inlet end of the piston and push the frit out gently. If the frit does not dislodge easily, the piston assembly can be submerged in hot water (maximum of 120°C) to expand plastic and allow for easier frit removal.

**NOTE:** Stainless Steel frits cannot be changed.

To reinsert a frit, in addition to the piston and frit an appropriate frit insertion tool must be used. The frit insertion tool should be dismantled into constituent parts. The open-ended piece is placed on top of the piston. One side will not fit correctly, so use the side the slides into the frit insertion tool smoothly. The piston needs to be in the orientation where the frit side is inside the tool. Once the open-ended part of the frit insertion tool is on the piston, place the frit into the open side of the frit insertion tool.

It is important to note that there is a specific orientation to the frit, and the frit should be installed larger diameter first, ensuring it locks in place. See diagram below.





# CHEMICAL COMPATIBILITY

Chemical compatibility information relates to components within the wetted flow path only and is based on generic information regarding the materials of construction.

Green indicates the material is compliant in the table, limited compliance yellow, and incompatibility red.

Unless specified the chemical concentration is 100% and exposure duration is 48hrs

| | Piston | | Frit | | O-Ring | |
|---|---|---|---|---|---|---|
| **Chemical** | **Acetal** | **PEEK** | **PE** | **SS** | **Viton** | **Kalrez** |
| **1,2-dichloroethane** | | | | | | |
| **Acetic acid 25%** | | | | | | |
| **Acetone 5%** | | | | | | |
| **Acetonitrile** | | | | | | |
| **Ammonia aqueous<25%** | | | | | | |
| **Ammonium Sulphate 10-40%** | | | | | | |
| **Benzyl alcohol 1%** | | | | | | |
| **Benzyl alcohol 2%** | | | | | | |
| **Butanol** | | | | | | |
| **Calcium chloride ($CaCl_2$)** | | | | | | |
| **Calcium hydroxide 30%** | | | | | | |
| **Calcium hypochlorite** | | | | | | |
| **Chloroacetic acid 50%** | | | | | | |
| **Chloroform** | | | | | | |
| **Chromic acid 10%** | | | | | | |
| **Citric acid** | | | | | | |
| **Copper sulphate** | | | | | | |
| **Dichloromethane** | | | | | | |
| **Dimethyl formamide** | | | | | | |

| | Piston | | Frit | | O-Ring | |
|---|---|---|---|---|---|---|
| Chemical | Acetal | PEEK | PE | SS | Viton | Kalrez |
| Dimethyl sulfoxide 10% | 🟥 | 🟨 | 🟩 | 🟩 | 🟥 | 🟩 |
| Disodium phosphate | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 |
| Ethanol 20% | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 |
| Ethanol 70% | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 |
| Ethylene glycol (1,2-ethanediol) | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 |
| Formaldehyde 50% | 🟩 | 🟩 | 🟩 | 🟩 | 🟥 | 🟩 |
| Glycerol (Glycerine) | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 |
| Guanidine hydrochloride 6M | 🟩 | 🟩 | 🟩 | 🟥 | 🟩 | 🟩 |
| Hydrochloric acid 10% | 🟥 | 🟩 | 🟩 | 🟥 | 🟩 | 🟩 |
| Hydrofluoric acid | 🟥 | 🟥 | 🟩 | 🟥 | 🟩 | 🟩 |
| Hydrogen peroxide | 🟥 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 |
| Industrial methylated spirit (IMS) | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 |
| Methanol 20% | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 |
| Methanol 50% | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 |
| Methyl chloride (Chloromethane) | 🟩 | 🟩 | 🟨 | 🟩 | 🟥 | 🟩 |
| Methyl ethyl ketone (MEK) | 🟩 | 🟩 | 🟩 | 🟩 | 🟥 | 🟩 |
| Methylene chloride | 🟨 | 🟩 | 🟨 | 🟩 | 🟥 | 🟩 |
| Nitric acid 10% | 🟥 | 🟩 | 🟩 | 🟩 | 🟥 | 🟩 |
| Nitric acid 70% | 🟥 | 🟥 | 🟩 | 🟩 | 🟥 | 🟩 |
| Peracetic acid 300 ppm | 🟥 | 🟩 | 🟩 | 🟩 | 🟨 | 🟩 |
| Phosphoric acid | 🟥 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 |
| Potassium hydroxide 2M | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 |

| | Piston | | Frit | | O-Ring | |
|---|---|---|---|---|---|---|
| Chemical | Acetal | PEEK | PE | SS | Viton | Kalrez |
| Iso-propanol 20% | | | | | | |
| Iso-propanol 40% | | | | | | |
| n-propanol 20% | | | | | | |
| Sodium acetate | | | | | | |
| Sodium bicarbonate 20% | | | | | | |
| Sodium carbonate | | | | | | |
| Sodium chlorate | | | | | | |
| Sodium chloride 2M | | | | | | |
| Sodium chloride 6M | | | | | | |
| Sodium hydroxide 0.5M | | | | | | |
| Sodium hydroxide 2M | | | | | | |
| Sodium hypochlorite 200 ppm | | | | | | |
| Sodium nitrate | | | | | | |
| Sodium sulphate | | | | | | |
| Trichloroethene (Trichloroethylene) | | | | | | |
| Urea 6M | | | | | | |
| Zinc chloride | | | | | | |

# TROUBLESHOOTING

| Problem | Cause | Solution |
|---|---|---|
| **Air pockets** | Solvent evaporation or gas evolution during storage | Recondition the column |
| **Abnormal pressure fluctuations during operation** | Incorrect valve position | Check valve position |
| | Blocked frit | Remove and dismantle piston, replace frit, reassemble and re-insert piston<br>Recondition column |
| | Fitting tightened too much | Replace fittings and ferrules, re-cut the end of the tubing |
| **Column leaking solvents** | Leaking piston | Visual check: is solvent present over the O-ring?<br>Remove piston from glass body, clean and replace O-ring |
| | Leaking tubing connection | Tighten fittings to chromatography system<br>Check tubing and connectors for leaks; replace if necessary |
| **Deteriorated peak shape of eluted substance** | Bead bed mechanically damaged | Repack column |
| | Blocked frit | Remove and dismantle piston, replace frit, reassemble and re-insert piston<br>Recondition column |
| | Dead volume at column inlet | Rotate the SNAP® clamp counterclockwise until piston just contacts bed |
| | Contamination affecting separation efficiency of stationary phase | Repack column in sterile environment |
| **Pressure drops during operation** | Tubing or fitting leak between pump and column | Check tubing/connections |
| | Solvent supply is dry | Refill solvent |

**If further troubleshooting is needed, please reach out to techsupport@astrea-bio.com.**

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Issue Date: 09 October 2024
CCR Number: N/A Process-ready columns added, and operating instructions clarified
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QA Reviewer Name: T Moore



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