Bio-Works

INSTRUCTION

# GoBio Prod Columns

## Safety

Please abide by following handling recommendations below. Please read the associated Safety Data Sheets (SDS) for the packed resin.

## Column handling on delivery

Open the box from the top. Carefully take out the product boxes. The side with the product label should face upwards. Open from the top of the product box, remove the protection material. Carefully hold the top side of the bag to take the column out, for larger columns two people may be required as this column is very heavy. Be careful not to damage the tubing connections on the top.

Place the bagged column into a cold room on delivery.  Make sure the column doesn't fall over during the storage period.

## Introduction

GoBio™ Prod columns are designed to perform chromatography purification of biological molecules in either GMP or non-GMP applications. The GoBio Prod prepacked column platform offers an alternative to conventional "pack-in-place" glass or stainless steel columns and is reliably packed with any WorkBeads™ resin. To accommodate a wide range of applications, GoBio Prod columns are configurable for nearly any bed height and industry standard internal diameter.

## Regulatory Support File

For additional details on the design of the GoBio Prod product line and relevant quality parameters, refer to the Regulatory Support File (RSF). The RSF can be requested by emailing info@bio-works.com.

# General information of the column construction

All the column components that are in contact with fluids meet the regulatory requirements e.g. FDA CFR 177, USP Class VI and animal origin free (or in compliance to the EMEA 410/01). The detailed information is available in the regulatory support file of GMP grade columns.

**Column body** (i.e. the tube): Made of borosilicate glass (26/50 mm ID) or acrylic (≥ 80 mm ID).

**End plunger:** The main body, the flow distributor and the triclamp connector are made of polypropylene. The o-ring is of viton rubber. The triclamp gasket is made of EPDM. The supporting mesh is made of polyamide of 15 micrometre. They are inert to most aqueous buffers.

**Connection:** Both ends of the column have standard ½" – ¾" triclamp connector. The End Cap is made of polypropylene. The triclamp is made of nylon.

**Holding frame:** M8 stainless steel

**Operating pressure:** Recommended rating is up to 3 bar (or 0.3 MPa, or 42 psi).

**Operating temperature:** 4°C to 30°C is recommended.

Note: If the column is used at cold room temperatures flow rates must be adapted for the increase in viscosity so that the maximal back pressure (5 Bar) over the column isn't exceeded.

**Inlet and outlet arrangement:** The inlet and outlet of the column are both located on the top of the column. The outlet of the column is located at the center of the column top and is sealed with a triclamp End Cap. The inlet of the column is located at the side. Fluid is introduced from the bottom of the column. It is connected to a soft sealing tubing on delivery to prevent the column from getting dry.

**Table 1.** Physical specifications.

| | Column inner diameter | | | | | |
|---|---|---|---|---|---|---|
| **Physical attributes** | **26 mm** | **50 mm** | **80 mm** | **100 mm** | **129 mm** | **240 mm** |
| Internal cross section | 5.3 $cm^2$ | 19.6 $cm^2$ | 50 $cm^2$ | 78 $cm^2$ | 130 $cm^2$ | 452 $cm^2$ |
| Column body pressure rating | 5 bar | 5 bar | 5 bar | 5 bar | 5 bar | 5 bar |
| Bed height range | 0 to 1000 mm | | | | | |
| Column volumes: | | | | | | |
| 10 cm bed height | 53 mL | 0.2 L | 0.5 L | 0.8 L | 1.3 L | 4.5 L |
| 20 cm bed height | 106 mL | 0.4 L | 1.0 L | 1.5 L | 2.6 L | 9 L |
| 60 cm bed height | 318 mL | 1.2 L | 3.0 L | 4.5 L | 7.8 L | 27 L |
| Assembled column height (cm) | ~15 + bed height | ~20 + bed height | ~25 + bed height | ~30 + bed height | ~30 + bed height | ~33 + bed height |
| Inlet/outlet flow path internal diameter | 3.18 mm/0.125" | 3.18 mm/0.125" | 6.35 mm/0.25" | 6.35 mm/0.25" | 9.53 mm/0.375" | 9.53 mm/0.375" |
| Inlet and outlet port connectors | Triclamp | Triclamp | Triclamp | Triclamp | Triclamp | Triclamp |

**Table 2.** Column Weight Table (approximate weight of packed column).

| | Column inner diameter | | | | | |
|---|---|---|---|---|---|---|
| **Bed height** | **26 mm** | **50 mm** | **80 mm** | **100 mm** | **129 mm** | **240 mm** |
| 5 cm | 0.5 kg (1 lbs) | 2 kg (4 lbs) | 4 kg (9 lbs) | 6 kg (13 lbs) | 10 kg (22 lbs) | 14 kg (31 lbs) |
| 10 cm | 0.6 kg (1.2 lbs) | 2.5 kg (5 lbs) | 5 kg (11 lbs) | 8 kg (18 lbs) | 13 kg (29 lbs) | 18 kg (40 lbs) |
| 15 cm | 0.8 kg (1.6 lbs) | 3 kg (7 lbs) | 6 kg (13 lbs) | 10 kg (22 lbs) | 6 kg (35 lbs) | 22 kg (48 lbs) |
| 20 cm | 1 kg (2 lbs) | 3.5 kg (8 lbs) | 7 kg (15 lbs) | 12 kg (26 lbs) | 19 kg (42 lbs) | 26 kg (57 lbs) |
| 60 cm | 3 kg (7 lbs) | 4 kg (9 lbs) | 9 kg (20 lbs) | 16 kg (35 lbs) | 24 kg (53 lbs) | 34 kg (75 lbs) |

## Column Packing and Storage

Each column is packed, tested, sanitized and sealed inside a Class 7 cleanroom. The column is sanitized using 0.5 M NaOH and stored in 20% ethanol.

After labelling, the column is sealed inside 2 heat-sealed polyolefin bags in the clean room.

## Instruction of Use

The column should be stored in a cold room after it is delivered. Handle the column with care to avoid any damage, as the column connections are vulnerable to strong forces.

Check the bioburden and endotoxin level of the column before use. If the level is too high, carry out the sanitization-in-place step before use.

**Caution:** Be extremely careful during the tightening and loosening of the outlet triclamp. Hold the triclamp firmly under balanced force when unscrewing or screwing its knob, as the triclamp connection to the column could be broken under a non-balanced force. The whole column will be ruined if the outlet triclamp connection is broken. It is not repairable.

1. Remove the protection bags with care.
2. Place the column upwards to a suitable surface in a vertical position.
3. Hold the flexible sealing tubing upwards. Gently tap its lower side and the upper side of the inlet tubing. It will allow air bubbles in the inlet tubing of the column (if any) to escape.
4. Gently remove the triclamp between the sealing tubing and the inlet. Gently squeeze the sealing tubing first, then disconnect it from the inlet. This procedure avoids sucking air into the inlet tubing.
5. Connect the inlet of the column to the chromatography system. Be careful not to trap air bubbles, for example, by running the instrument at very low flow rate during the connection period.
6. Very carefully remove the outlet triclamp and the end cap. Connect the outlet to the chromatography instrument.
7. Check for the bioburden and endotoxin level. Alternatively, go to step 8.
8. Sanitization-in-place (SIP) step. Run 1 column volumes (CV), of 0.5 M NaOH and 4 CV, of the equilibration buffer at a flow rate of about 60 cm/h, make sure that the maximal back pressure for the column (5 Bar) isn't exceeded. The flow rate can be increased after 2 CV of the equilibration buffer is pumped through as long as the maximal back pressure (5 Bar) isn't exceeded.
9. The column is ready for use.

## Storage after use

For the short-term storage, disconnect the outlet side of the column and seal it with a triclamp end cap. Then disconnect the inlet of the column and seal it with a triclamp end cap. Make sure the end caps are sanitized before use.

For the long-term storage, follow the instructions below.

1. Equilibrate the column with the storage liquid. If an organic solvent such as 20% ethanol is used, introduce it in a reduced flowrate such as 60 cm/h, make sure that the maximal back pressure for the column (5 Bar) isn't exceeded.
2. Disconnect the outlet of the column. Seal it using a Triclamp end cap.
3. Disconnect the inlet of the column from the chromatography system.
4. Connect one end of the sealing tubing to the inlet tubing of the chromatography system.
5. Hold the sealing tubing upwards. Run the pump slowly to fill the sealing tubing with the storage liquid. Then connect it to the inlet of the column. Avoid trapping air bubbles.
6. Disconnect the sealing tubing from the chromatography system.
7. Seal it using an end cap.
8. Place the column to a cold room.

## Troubleshooting

**Air in the column**

If air entered the inlet port and did not reach the column (to the best assessment of the operator), attach a 3-way valve to the column inlet and the other end to the chromatography system. Leave the column outlet closed, see Figure 1.

Attach a syringe to the purge line while pumping the mobile phase at low flow rate (i.e. 50 cm/h), 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.

After all air has been purged for the inlet line, change the direction of the valve as indicated in Figure 2 below. With the flow off open the column outlet and connect it to the chromatography system. Introduce flow to the column at low flow rate (i.e. 50 cm/h) to flush trapped air from the columns outlet.

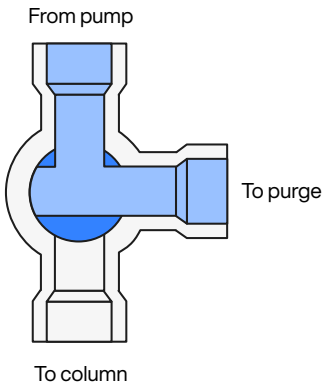


**Figure 1.** Valve in purge position.

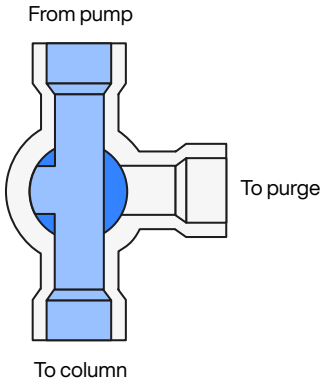


**Figure 2.** Valve in column position.

If air entered the packed chromatography bed, recondition the column by running a solution with low surface tension (i.e. 1% surfactant (e.g. Tween) or 20% ethanol) in reverse flow for 2 – 3 CV. Increased backpressure on the column outlet may aid in forcing air bubbles out from the solution.

Retest the column performance (efficiency and asymmetry) according to the instructions below.

## High pressure during the first use of the column

Potential causes to high pressure include:

- Operation under higher flow than recommended for the packed resin bed
  - Flow of alcohols (e.g. storage solution) through the column is known to increase column pressure
  - Reduce the flow rate through the column to abide to the pressure limit of the packed resin bed
  - Confirm that high viscosity solutions are not being used during pressure evaluation
- Temperature shifts between buffers used in the column
  - Allow all buffers and the column to equilibrate to ambient temperatures
- Undersized tubing, fittings and/or gaskets
  - Refer to table 1 for flow path sizing
- Incorrect column valve position
  - Check valve position
- Flow path restriction
  - Check for blockage

## Pressure increase during run

Potential causes to high pressure include:

- Product or precipitates clogging the of the mesh
  - Clean the column with the appropriate cleaning method for the residue that clogged the mesh and/or resin. Running in reverse flow mode is recommended
  - Flow >5 CV of equilibration buffer through the column in reverse flow. Recheck pressure and column performance (efficiency, asymmetry, pressure vs. flow) under normal operating conditions for comparison to results on the CoA
- Operation under higher flow than recommended for the packed resin bed
  - Reduce the flow rate through the column to abide to the 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
- Residue build up at the inlet of the column
  - Flow >5 CV of equilibration buffer through the column in reverse flow. Recheck pressure and column performance (efficiency, asymmetry, pressure vs. flow) under normal operating conditions for comparison to results on the CoA
- Use of high viscosity solutions or high product load concentrations
  - Limit the use of high viscosity solutions and decrease the product load concentrations
- Fouled chromatography resin
  - Clean the column with the appropriate cleaning method for the residue that clogged the resin. Running in reverse flow mode is recommended
  - Flow >5 CV of equilibration buffer through the column in reverse flow. Recheck pressure and column performance (efficiency, asymmetry, pressure vs. flow) under normal operating conditions for comparison to results on the CoA
- Temperature shifts between buffers used in the column
  - Allow all buffers and the column to equilibrate to ambient temperatures

## Pressure drop during run

Potential causes to high pressure include:

- Line or fitting leaks
  - Check all lines and connections
- Temperature shifts between buffers used in the column
  - Allow all buffers and the column to equilibrate to ambient temperatures
- Viscosity shifts during buffer transitions
  - Allow the column to equilibrate

## Column performance testing

Follow the steps below to measure the plate count and asymmetry of you GoBio Prod column. Please note that minor differences (+/- 10 – 20%) in the measured plate count and asymmetry noted on the column CoA are to be expected.

Sources of variation includes

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

Method:

1. Remove column storage solution, run equilibration buffer at low flow rate (i.e. 50 cm/h) for 2 – 3 CV
2. Condition the column with the equilibration buffer for 1 – 2 CV at column qualification testing flow rate.
3. Conduct a pulse injection with 1 – 2% CV of the injection solution
4. Elute with mobile phase for 1 – 2 CV at the same test flow rate while monitoring UV or conductivity depending on the injection solution
   a. Salt injections are typically analyzed with a conductivity meter while acetone injection solutions are analyzed with a UV meter.
   b. Calculate number of theoretical plates and asymmetry of the eluted peak:

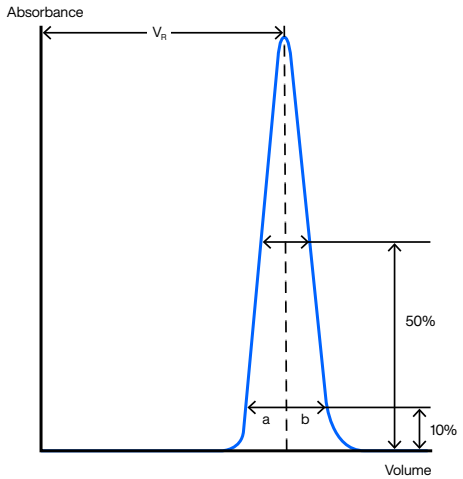


**Figure 3.** Absorbance graph.

Theoretical plates:

$$N = 5.54 \times \left(\frac{V_R}{W_h}\right)^2$$

Where:

N = number of theoretical plates
$V_R$ = peak retention (elution) volume
$W_{½}$ = peak width at half height

Asymmetry:

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

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

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Bio-Works, Virdings allé 18, 754 50 Uppsala, Sweden. For local office contact information, visit bio-works.com/contact.

IN 55 410 042 BA

Bio-Works