

Tricorn Empty High Performance Columns

Instructions

Introduction

Tricorn™ columns are designed for high resolution liquid chromatography of biomolecules performed at medium to high pressures (see Specifications). They are suitable for use with high performance media, such as Sepharose™ High Performance, Superdex™ Prep Grade and SOURCE™.

When using Tricorn columns for large volume or repeated loading of clarified feed in combination with capture resins, such as Sepharose Fast Flow or High Flow Agarose, the Tricorn coarse filter kit is recommended to reduce the risk of clogging.

When using Tricorn columns for polyclonal IgG or monoclonal antibody purification in combination with resins, such as MabSelect pcc or MabSelect Prisma, the Tricorn Medium Filter Kit is recommended to reduce the risk of clogging.

The columns are available with inner diameters of 5 and 10 mm, and in lengths from 20 to 200 mm. Column lengths of 300 and 600 mm are also available for the 10-mm diameter columns. The design and dimensions of the columns make them particularly useful for screening and polishing steps in protein purification.

When you receive your column

Check that your column package contains the following:

- Column tube
- Adaptor unit
- End cap
- Filter Kit including adaptor and bottom filters, and O-rings
- Two stop plugs, two fingertight connectors, two M6 connectors, adaptor lock and filter holder

If any item is missing or appears to be damaged, contact your local Cytiva office.

System compatibility

Tricorn columns are designed to be used with ÄKTA™ and FPLC™ systems. Other systems using M6 or 1/16-inch connectors can also be used.

Technical description

Column tube: High-precision plastic-coated borosilicate glass tube. The plastic coating keeps the glass in place if a breakage occurs. PEEK (polyetheretherketon) plastic threaded fittings on both ends connect to the adaptor unit and the bottom unit respectively. Small quantities of air can occasionally be trapped between the glass and the coating film during manufacture. The resulting uneven surface does not affect column performance or durability.

Bottom unit: Black polypropylene cap holds filter holder in place in the bottom of the column tube. The filter holder is fitted with an O-ring seal and is keyed so that it cannot rotate within the column tube.

Adaptor unit: Red polypropylene unit containing an inner locking device and an adapter that fits into the top of the column tube. There is an adapter lock on the top of the adapter unit that prevents accidental adjustment of the resins bed height. The adapter lock should always be in the locked position (pressed down) except when adjusting the position of the adapter. The adapter is fitted with an O-ring seal and is keyed so that it cannot rotate within the column tube when locked. A filter (included in the column package) is placed between the adapter and the resin bed.

First-time use

When using the column for the first time, you should rinse all parts in 20% ethanol, and reassemble the column before packing it with resin. A chromatography system should be connected for packing, packing evaluation, and chromatography.

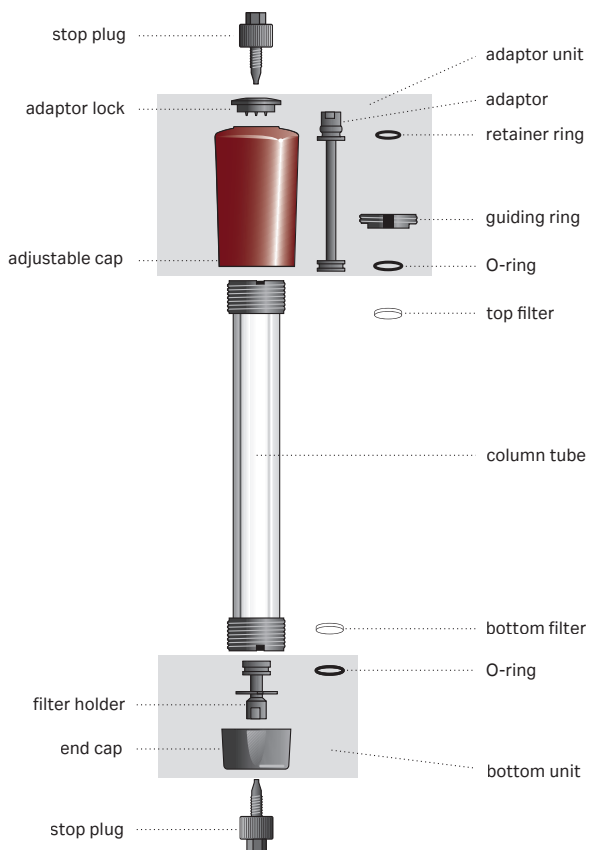


Fig 1. Tricorn empty column

Assembling the column

To assemble the column, proceed as follows:

Note: The adapter unit is located at the top of the column.

Step	Action
1	Insert a bottom filter (Figure above) into the filter holder.
2	Wet the O-ring on the filter holder by dipping the filter holder into water, buffer, or 20% ethanol.
3	Insert the filter holder into the column tube. Make sure that the keyed part of the filter holder fits into the slot on the threaded section on the column tube. Push the filter holder into place.
4	Screw the end cap onto the column tube..

Packing the column

Before starting to pack your Tricorn column, refer to the packing instructions included with the chromatographic resin that you intend to use.

To fill the empty column, you can use Tricorn Packing Equipment, which is a complete column packing set-up as shown in the [Figure 2, on page 2](#). Alternatively, you can purchase each packing component separately to make your own packing set-up. The advantage of doing this is that you can choose a Tricorn Packing Connector and Tricorn Glass Tube to suit your application.

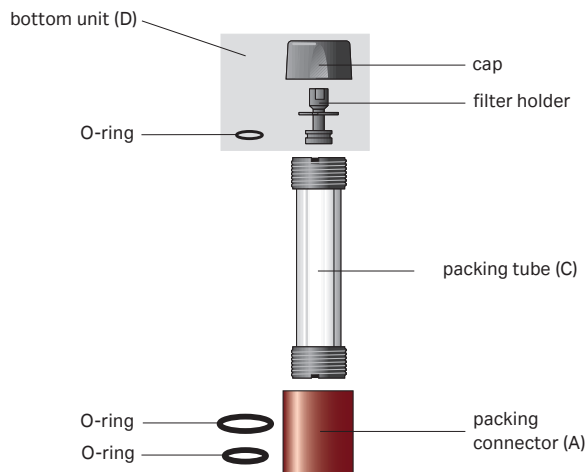


Fig 2. Tricorn Packing Equipment.

To pack the column, proceed as follows:

Step	Action
1	Screw a suitable Tricorn Packing Connector (accessory, Figure above, A) onto the top of the column tube. The Tricorn Packing Connector must be fitted with suitable O-rings (included with the Tricorn Packing Connector).
2	Place the filter holder into the bottom of the column tube and secure it with the cap. Make sure the inner locking device keys with the slot on the column tube threads (Figure 3, on page 3, A). Insert a stop plug into the bottom unit.
3	Screw the Tricorn Packing Tube (accessory, Figure above, C) into the upper fitting of the Tricorn Packing Connector. The dimensions of the Tricorn Packing Tube depend on the chromatography resin being used.
4	Calculate how much chromatography resin is necessary as described in the instructions for the resin being used.
5	Pour the chromatography resin into the top of the packing tube filling both column tube and packing tube. Remove all air bubbles.
6	Attach the bottom unit (Figure above, D) to the top of the packing tube. Place a beaker beneath the column tube and remove the stop plug from the end cap of the column tube.
7	Connect a pump to the top of the packing unit.
8	Pack the column by pressure or flow, depending on the instructions included with the chromatography resin.
9	When the resin is packed, switch off and disconnect the pump, re-fit the stop plug into the cap of the column tube and remove the packing tube and packing connector.
10	Top off the column with the same fluid as used for packing the column.
11	Place a pre-wet top filter on top of the fluid in the column.

Note:

The top coarse filter and the top medium filter are inserted by another procedure. See separate instruction included in the respective filter kit.

Step	Action
12	Screw the guiding ring (Figure below, A) inside the adapter unit down to its end position so that it is level with the bottom of the adapter unit, see Figure below..
13	Wet the O-ring on the adapter unit by dipping it into water, buffer or 20% ethanol.
14	Screw the guiding ring back 1.5 turns.
15	Screw the adapter unit onto the column tube, ensuring the inner part of the guiding ring fits into the slot on the column tube threads. (Figure below, B) Make sure that there are no air bubbles.
16	Screw the adapter halfway down. Press the adapter lock down into the locked position. It should now not be possible to turn the adapter if the adapter unit has been mounted correctly.

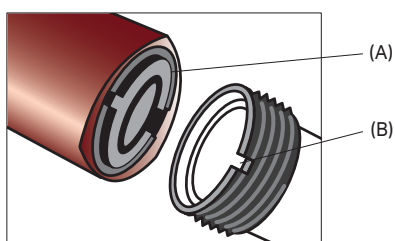


Fig 3. Adapter unit and slot on column tube threads.

- 17 Unlock the adapter lock and screw the adapter unit down until the adapter meets the resin bed surface. Screw the adapter unit down a further 360 degrees so that the adapter is positioned slightly below the resin surface.
- 18 Press the adapter lock down into the locked position.
- 19 Screw a stop plug into the adapter unit. The column is now ready for use or storage.

Note:

Although it is possible to fit the adapter unit on the column tube without keying the inner locking device into the slot on the column tube, the adapter lock will not function. The consequence of this is that the adapter is not locked in position and accidental turning of the adapter is possible.

Connecting the column to a system

To connect the Tricorn column to a system:

Step	Action
1	Mount the column in a vertical position with the adapter unit uppermost.
2	Make sure the inlet capillary tubing from the system contains fluid. The system should deliver a flow of about 0.5 mL/min while the column is being connected.
3	Remove the stop plug from the adapter unit.
4	Connect the inlet capillary tubing from the system. For the ÄKTA system, the inlet capillary tubing can be fitted directly into the adapter unit. For other systems using M6 connectors, an M6 female adapter is necessary (included in the column package).

Step	Action
5	Remove the stop plug from the bottom unit immediately after connecting the inlet capillary tubing.
6	Connect the outlet capillary tubing.
7	Wash and equilibrate the column as required.

For further information, refer to the instrument manuals for the system being used.

Maintenance

Dismantling and cleaning the column and parts

To dismantle the column, proceed as follows (numbers in parantheses refer to [Figure 1, on page 2](#)):

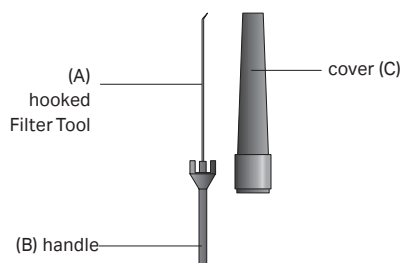
Step	Action
1	Lift the adapter lock (Figure 1, on page 2, B) one "click" to the unlocked position.
2	Unscrew the adapter unit (Figure 1, on page 2, A).
3	Unscrew the end cap (Figure 1, on page 2, M) and remove it from the column tube (Figure 1, on page 2, I). The filter holder (Figure 1, on page 2, L) is now visible with the filter in place.
4	Screw a stop plug (included in the column package) into the filter holder as a tool to remove the holder. Pull the filter holder out carefully. If resistance is encountered, fill the column with a small amount of water.
5	Unscrew the stop plug from the filter holder.
6	If necessary, clean the column and its constituent parts in solutions of laboratory detergents. Enzyme detergents are recommended for removing contaminating proteins.
7	Wash the parts thoroughly in distilled water before reassembling the column.
8	Reassemble the column as described previously.

Replacing the top filter

Before replacing the top filter, try cleaning the column resin first as recommended in the instructions supplied with the resin. Replace the top filter if you still observe increased back-pressure, a loss of resolution or sample recovery, after cleaning the resin *in situ*, or when cleaning the column.

If the top filter is clogged and needs to be replaced and/or the resin bed surface becomes contaminated, proceed as follows:

Step	Action
1	Disconnect the column from the system and insert a stop plug into the bottom unit.
2	Lift the adapter lock and remove the adapter unit.
3	Using a hooked Filter Tool (accessory Figure 4, on page 4, A), carefully remove the filter from the surface of the resin bed.

Step Action**Fig 4.** Filter tool.

- 4 Fill the column tube with fluid and carefully stir the top 1 mm of the resin bed surface with a Pasteur pipette and remove the suspended resin particles.
- 5 Top off the column tube with fluid and remove all air bubbles.
- 6 Place a new pre-wet top filter on top of the fluid in the column.

Note:

The top coarse filter and the top medium filters are inserted by another procedure. See separate instruction included in the respective filter kit.

- 7 Screw the adapter unit onto the column tube, ensuring that the inner part keys with the slot on the column tube threads (Figure 3, on page 3, A).
- 8 Screw the adapter unit down until the adapter meets the resin bed surface.
- 9 Screw the adapter unit down a further 360 degrees so that the adapter is positioned slightly below the resin bed surface.
- 10 Press the adapter lock into the locked position.
- 11 The column is now ready for use.

If the column is not to be used immediately, fit in a stop plug into the adapter unit.

Note:

Do not tighten the adaptor unit using a wrench. Tighten by hand only.

Replacing the bottom filter/unpacking the column

We do not recommend changing the bottom filter without repacking the column as this can lead to a loss of efficiency. This will also mean that the top filter should be replaced. To replace the bottom filter, proceed as follows:

Step Action

- 1 Disconnect the column from the system.
- 2 Lift the adapter lock and remove the adapter unit. Bring up the loose filter using the hooked Filter Tool (Figure 4, on page 4, A).
- 3 Invert the column and attach to a stand over a flask that will contain the expelled resin.
- 4 Connect a pump to the bottom unit.
- 5 Start the pump at a flow rate of 5 mL/min. When the resin starts moving down the column, the flow rate can be increased. Monitor the pressure if possible to make sure that the resin and/or the column are not being over pressured.

Step Action

- 6 Disconnect the column from the pump and dismantle and clean the column as described previously.
- 7 Insert a new bottom filter into the filter holder and press it into place using the handle of the Filter Tool (Figure 4, on page 4, B) or the smaller end of the Filter Tool cover (Figure 4, on page 4, C). The handle of the Filter Tool is used for filter in 5-mm columns, the smaller end of the Filter Tool cover for filters in 10-mm columns.
- 8 Reassemble and repack the column as described previously.

Replacing O-rings

There are O-rings in the adapter unit and in the bottom filter holder. Before replacing an O-ring, make sure that you have a replacement O-ring of the correct size and type.

To remove and replace the used O-rings, gently use a pair of forceps, taking care not to damage the O-ring fitting or housing. To facilitate the removal of the used O-ring, it is recommended to use the FPLC Wrench (accessory, see Ordering information). With gentle force, place the plastic wrench over the O-ring causing the O-ring to stretch out from the groove on the adapter. Use a pair of forceps to carefully remove the used O-ring from the fitting.

Moisten a new O-ring with water to aid fitting.

Note: *When replacing the O-ring, make sure that the new O-ring is not twisted when it is in position. A twisted O-ring can cause leakage.*

Chemical resistance

Tricorn glass columns can be used with aqueous buffer solutions and detergents at pH conditions between 1 and 14, and with nearly all organic solutions commonly used in chromatography. It is important, however, to consider the chemical compatibility and resistance of the chromatography resin packed in the column. Examples of chemicals that can be used in Tricorn glass columns are listed below. Solvent-resistant O-rings (PFR) should be mounted in the column's bottom and adapter units when using organic solvents (listed below in italics)

Acetic acid
Acetonitrile
Butanol
 Citric acid
 CHAPS
Dimethylformamide (DMF)
Dimethylsulfoxide (DMSO)
 Ethanol
 Ethylenediaminetetraacetic acid (EDTA)
Formaldehyde (40%)
Formic acid
Glycerol
 Guanidine HCl (6 M)
 Hydrochloric acid (2 M)
Isopropanol
 Lactic acid

Methanol

Phosphoric acid

Perfluoroalkoxyl (PFPA)

Sodium dodecyl sulfate (SDS)

Sodium acetate (saturated)

Sodium hydroxide (2 M)

Sodium thiocyanate (3 M)

Sulfuric acid (dilute)

Trifluoroacetic acid (0.1%)

TWEEN™

Urea (8 M)

Under operating conditions, the following inert materials are in contact with the eluent: PEEK (filter holder), borosilicate glass, PE (filter), and EPDM. EPDM O-rings can be replaced with optional PFR O-rings that are more solvent-resistant.

Technical Specifications

Materials in contact with eluent	Borosilicate glass, PEEK, PP, EPDM and PFR ¹
Column dimensions (inner diameter × length), mm	5 × 20, 5 × 50, 5 × 100, 5 × 150, 5 × 200, 10 × 20, 10 × 50, 10 × 100, 10 × 150, 10 × 200, 10 × 300, 10 × 600
Max operating pressure	
Tricorn 5	10 MPa, (100 bar, 1450 psi)
Tricorn 10	5 MPa, (50 bar, 725 psi)
Temperature	
Operating	4°C to 40°C

¹ PE = polyethylene

PEEK = polyetheretherketone

EPDM = ethylene propylene diene monomer

PFR = perfluoro-rubber

Bed Volumes and Heights

Tricorn Column	Column Size i.d. (mm)	With one adapter		With two adapters	
		Volume (mL)	Bed Height (mm)	Volume (mL)	Bed Height (mm)
Tricorn 5/20	5	0.10–0.57	5–29	0.00–0.52	0–26
Tricorn 5/50	5	0.69–1.16	35–59	0.16–1.11	8–56
Tricorn 5/100	5	1.67–2.14	85–109	1.15–2.09	58–106
Tricorn 5/150	5	2.65–3.12	135–159	2.13–3.07	108–156
Tricorn 5/200	5	3.63–4.11	185–209	3.11–4.05	158–206
Tricorn 10/20	10	0.00–2.29	0–29	0.00–2.07	0–26
Tricorn 10/50	10	2.29–4.64	29–59	0.00–4.43	0–56
Tricorn 10/100	10	6.21–8.57	79–109	3.64–8.36	46–106
Tricorn 10/150	10	10.14–12.50	129–159	7.57–12.28	96–156
Tricorn 10/200	10	14.07–16.42	179–209	11.50–16.21	146–206
Tricorn 10/300	10	21.92–24.28	279–309	19.35–24.06	246–306
Tricorn 10/600	10	45.48–47.84	579–609	42.91–47.63	546–606

Ordering information

Columns

Product	Quantity	Product code
Tricorn 5/20 column	1	28406408
Tricorn 5/50 column	1	28406409
Tricorn 5/100 column	1	28406410
Tricorn 5/150 column	1	28406411
Tricorn 5/200 column	1	28406412
Tricorn 10/20 column	1	28406413
Tricorn 10/50 column	1	28406414
Tricorn 10/100 column	1	28406415
Tricorn 10/150 column	1	28406416
Tricorn 10/200 column	1	28406417
Tricorn 10/300 column	1	28406418
Tricorn 10/600 column	1	28406419
Tricorn packing connector 5-5 ¹	1	18115321
Tricorn packing connector 5-10 ¹	1	18115322
Tricorn packing connector 10-10 ¹	1	18115323
Tricorn packing equipment 5/50 ²	1	18115324
Tricorn packing equipment 10/100 ²	1	18115325

¹ Tricorn packing connector includes the packing connector with 2 EPDM O-rings.

² Tricorn packing equipment includes the Packing equipment 5/50 (5 mm connector and 50 mm glass tube) or 10/100 (10 mm connector and 100 mm glass tube), EPDM O-rings and bottom unit and stop plug (Fig 2).

Accessories

Product	Quantity	Product code
Tricorn 5/20 glass tube	1	18115304
Tricorn 5/50 glass tube	1	18115305
Tricorn 5/100 glass tube	1	18115306
Tricorn 5/150 glass tube	1	18115307
Tricorn 5/200 glass tube	1	18115308
Tricorn 10/20 glass tube	1	18115313
Tricorn 10/50 glass tube	1	18115314
Tricorn 10/100 glass tube	1	18115315
Tricorn 10/150 glass tube	1	18115316
Tricorn 10/200 glass tube	1	18115317
Tricorn 10/300 glass tube	1	18115318
Tricorn 10/600 glass tube	1	18115319
Tricorn 5 adapter unit	1	28406406
Tricorn 5 bottom unit	1	18115301
Tricorn 10 adapter unit	1	28406407
Tricorn 10 bottom unit	1	18115310
Tricorn 5 filter kit ¹	1	29053586
Tricorn 10 filter kit ¹	1	29053612
Tricorn 5 coarse filter kit ¹	1	11001253
Tricorn 10 coarse filter kit ¹	1	11001254
Tricorn 5 Medium Filter Kit ¹	1	29258132
Tricorn 10 Medium Filter Kit ¹	1	29258131
Filter Tool	1	18115320
Tricorn O-ring packing kit ²	1	18115282
Solvent resistant O-rings		
O-ring 3 × 1 PFR (5-mm columns)	2	18115303
O-ring 7 × 1 PFR (10-mm columns and 5-mm packing connector)	2	18115312
O-ring 12 × 1 PFR (10-mm packing connector)	2	18115326
Fingertight connector, 1/16" male	10	18111255
FPLC wrench	1	19748101

¹ Do not store exposed to daylight. Both filter kits include top and bottom filters and EPDM O-rings, 5 of each.

² Tricorn O-ring packing kit includes O-ring 7×1 EPDM and O-ring 12×1 EPDM, 2 of each.

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