GE Healthcare

Instructions 28-4094-88 AA

High Performance Columns

Tricorn Empty

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[™].

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

When you receive your column

Please 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, please contact your local GE Healthcares' 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 ensures that the glass remains in place if a breakage occurs. PEEK (polyetheretherketon) plastic threaded fittings on both ends connect to the adaptor unit and the bottom unit respectively
- **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 adaptor that fits into the top of the column tube. There is an adaptor lock on the top of the adaptor unit that prevents accidental adjustment of the medium bed height. The adaptor lock should always be in the locked position (pressed down) except when adjusting the position of the adaptor. The adaptor is fitted with an Oring 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 adaptor and the medium 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 medium. A chromatography system should be connected for packing, packing evaluation, and chromatography

Assembling the column

To assemble the column, proceed as follows (numbers in parentheses refer to Fig 1): **Note:** The adaptor unit is located at the top of the column.



- ${\bf Fig 1.} \ {\rm Tricorn \ empty \ column.}$
- 1. Insert a bottom filter (Fig 1, J) into the filter holder (Fig 1, L)
- Wet the O-ring (Fig 1, K) on the filter holder by dipping the filter holder into water, buffer, or 20% ethanol.
- 3. Insert the filter holder into the column tube. Ensure 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 (Fig 1, M) onto the column tube.

Packing the column

Before starting to pack your Tricorn column, please refer to the packing instructions included with the chromatographic medium 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 Figure 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.

To pack the column, proceed as follows:

1. Screw a suitable Tricorn Packing Connector (accessory, Fig 2, A) onto the top of the column tube (Fig 1, I). The Tricorn Packing Connector must be fitted with suitable O-rings (included with the Tricorn Packing Connector).



 Place the filter holder (Fig 1, L) into the bottom of the column tube and secure it with the cap (Fig 1, M). Ensure the inner locking device keys with the slot on the column tube threads (Fig 3, A). Insert a stop plug (Fig 1, N) into the bottom unit.

- Screw the Tricorn Packing Tube (accessory, Fig 2, C) into the upper fitting of the Tricorn Packing Connector. The dimensions of the Tricorn Packing Tube depend on the chromatography medium being used.
- 4. Calculate how much chromatography medium is necessary as described in the instructions for the medium being used.
- 5. Pour the chromatography medium into the top of the packing tube filling both column tube and packing tube. Remove all air bubbles.
- 6. Attach the bottom unit (Fig 2, 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 medium.
- 9. When the medium 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 is inserted by another procedure. See separate instruction included in the coarse filter kit.
- 12. Screw the guiding ring (Fig 3, A) inside the adaptor unit down to its end position so that it is level with the bottom of the adaptor unit, see Fig 3.
- 13. Wet the O-ring (Fig 1, F) on the adaptor unit by dipping it into water, buffer or 20% ethanol.
- 14. Screw the guiding ring back 1.5 turns.
- 15. Screw the adaptor unit onto the column tube, ensuring the inner part of the guiding ring fits into the slot on the column tube threads. (Fig 3, B) Make sure that there are no air bubbles.
- 16. Screw the adaptor halfway down. Press the adaptor lock down into the locked position. It should now not be possible to turn the adaptor if the adaptor unit has been mounted correctly.
- 17. Unlock the adaptor lock and screw the adaptor unit down until the adaptor meets the medium bed surface. Screw the adaptor unit down a further 360 degrees so that the adaptor is positioned slightly below the medium surface
- 18. Press the adaptor lock down into the locked position.



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

- 19. Screw a stop plug into the adaptor unit. The column is now ready for use or storage.
- Note: Although it is possible to fit the adaptor unit on the column tube without keying the inner locking device into the slot on the column tube, the adaptor lock will not function. The consequence of this is that the adaptor is not locked in position and accidental turning of the adaptor is possible.

Pratical video instructions in good packing techniques are available on the interactive CD-ROM "Column Packing – The Movie", see ordering information.

Fig 2. Tricorn Packing Equipment.



Connecting the column to a system

To connect your Tricorn column to a system:

- 1. Mount the column in a vertical position with the adaptor unit uppermost.
- Ensure 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 adaptor unit.
- 4. Connect the inlet capillary tubing from the system. For the ÄKTA system, the inlet capillary tubing can be fitted directly into the adaptor unit. For other systems using M6 connectors, an M6 female adaptor is necessary (included in the column package).
- 5. Immediately after connecting the inlet capillary tubing, remove the stop plug from the bottom unit.
- 6. Connect the outlet capillary tubing.
- 7. Wash and equilibrate the column as required.

For further information, please refer to ordering information and Amersham Biosciences BioDirectory[™]. Also 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 Fig. 1):

- 1. Lift the adaptor lock (Fig 1, B) one "click" to the unlocked position.
- 2. Unscrew the adaptor unit (Fig 1, A).
- 3. Unscrew the end cap (Fig 1, M) and remove it from the column tube (Fig 1, I). The filter holder (Fig 1, 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.
- The column and its constituent parts can be cleaned 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

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

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

- 1. Disconnect the column from the system and insert a stop plug into the bottom unit.
- 2. Lift the adaptor lock and remove the adaptor unit.
- 3. Using a hooked Filter Tool (accessory Fig 4, A), carefully remove the filter from the surface of the medium bed.



Fig 4. Filter tool.

- Fill the column tube with fluid and carefully stir the top 1 mm of the medium bed surface with a Pasteur pipette and remove the suspended medium 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 is inserted by another procedure. See separate instruction included in the coarse filter kit.
- 7. Screw the adaptor unit onto the column tube, ensuring that the inner part keys with the slot on the column tube threads (Fig 3, A).



- 8. Screw the adaptor unit down until the adaptor meets the medium bed surface.
- 9. Screw the adaptor unit down a further 360 degrees so that the adaptor is positioned slightly below the medium bed surface.
- 10. Press the adaptor 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 adaptor 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 may lead to a loss of efficiency. This will also mean that the top filter should be replaced. To replace the bottom filter, please proceed as follows:

- 1. Disconnect the column from the system.
- 2. Lift the adaptor lock and remove the adaptor unit. Bring up the loose filter using the hooked Filter Tool (Fig 4, A).
- 3. Invert the column and attach to a stand over a flask that will contain the expelled medium
- 4. Connect a pump to the bottom unit.
- 5. Start the pump at a flow rate of 5 ml/min. When the medium starts moving down the column, the flow rate can be increased. Monitor the pressure if possible to ensure that the medium and/or the column are not being over pressured.
- 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 (Fig 4, B) or the smaller end of the Filter Tool cover (Fig 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 adaptor 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 adaptor. Use a pair of foreceps to carefully remove the used O-ring from the fitting.

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

Chemical resistance

Tricorn glass columns may 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 media 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 adaptor units when using organic solvents written in italics.

Acetic acid Acetonitrile Rutanol Citric acid CHAPS Dimethylformamide (DMF) Dimethulsulfoxide (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 dodecul sulfate (SDS) Sodium acetate (saturated) Sodium hydroxide (2 M) Sodium thiocyanate (3 M) Sulfuric acid (dilute) Trifluoroacetic acid (0.1%) Triton™ X-100 TWFEN™ Urea (8 M)

Under operating conditions, the following inert materials are in contact with the eluent: PEEK (filter holder), borosilicate glass, PP (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*		
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		
* PP = polypropylene			
PEEK = polyetheretherketone			
EPDM = ethylene propylene diene monomer			
DER - porfluoro rubbor			

Bed Volumes and Heights

Tricorn Column	Column Size i.d. (mm)	With one Volume (ml)	adaptor Bed Height (mm)	With two Volume (ml)	adaptors 3ed 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

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Product	Quantity	Code No.
Tricorn 5/20 column	1	28-4064-08
Tricorn 5/50 column	1	28-4064-09
Tricorn 5/100 column	1	28-4064-10
Tricorn 5/150 column	1	28-4064-11
Tricorn 5/200 column	1	28-4064-12
Tricorn 10/20 column	1	28-4064-13
Tricorn 10/50 column	1	28-4064-14
Fricorn 10/100 column	1	28-4064-15
Fricorn 10/150 column	1	28-4064-16
fricorn 10/200 column	1	28-4064-17
Fricorn 10/300 column	1	28-4064-18
fricorn 10/600 column	1	28-4064-19
Tricorn packing connector 5–5 *	1	18-1153-21
Tricorn packing connector 5–10 *	1	18-1153-22
Fricorn packing connector 10–10 *	1	18-1153-23
Tricorn packing equipment 5/50 **	1	18-1153-24
Fricorn packing equipment 10/100 **	1	18-1153-25

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

** 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 (Fia 2).

CD-ROM

www.gehealthcare.com/lifescience

GE Healthcare Bio-Sciences AB Björkgatan 30 SE-751 84 Uppsala Sweden



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Accessories

Product Tricorn 5/2

Product	Quantity	Code No.
Tricorn 5/20 glass tube	1	18-1153-04
Tricorn 5/50 glass tube	1	18-1153-05
Tricorn 5/100 glass tube	1	18-1153-06
Tricorn 5/150 glass tube	1	18-1153-07
Tricorn 5/200 glass tube	1	18-1153-08
Tricorn 10/20 glass tube	1	18-1153-13
Tricorn 10/50 glass tube	1	18-1153-14
Tricorn 10/100 glass tube	1	18-1153-15
Tricorn 10/150 glass tube	1	18-1153-16
Tricorn 10/200 glass tube	1	18-1153-17
Tricorn 10/300 glass tube	1	18-1153-18
Tricorn 10/600 glass tube	1	18-1153-19
Tricorn 5 adaptor unit	1	28-4064-06
Tricorn 5 bottom unit	1	18-1153-01
Tricorn 10 adaptor unit	1	28-4064-07
Tricorn 10 bottom unit	1	18-1153-10
Tricorn 5 filter kit*	1	18-1153-02
Tricorn 10 filter kit*	1	18-1153-11
Tricorn 5 coarse filter kit*	1	11-0012-53
Tricorn 10 coarse filter kit*	1	11-0012-54
Filter Tool	1	18-1153-20
Tricorn adaptor lock	2	18-1153-27
Tricorn O-ring packing kit**	1	18-1152-82
Solvent resistant O-rings		
O-ring 3 × 1 PFR (5-mm columns)	2	18-1153-03
O-ring 7 × 1 PFR (10-mm columns and		
5-mm packing connector)	2	18-1153-12
O-ring 12 × 1 PFR (10-mm packing connector)	2	18-1153-26
Fingertight connector, 1/16" male	10	18-1112-55
FPLC wrench	1	19-7481-01

* Both filter kits include top and bottom filters and EPDM O-rings, 5 of each.

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

Column Packing – The Movie 18-1165-33

For further information regarding accessories and chemicals, please see the latests **GE Healthcares BioDirectory**

Visit us at: www.gehealthcare.com/lifescience