GE Healthcare

Instructions 56-3241-43 AD

HR 16/5 column
HR 16/10 column
HR 16/50 column

Introduction

HR 16 columns are designed for high resolution liquid chromatography of biomolecules performed at low to medium pressures. The columns are suitable for use with high performance media such as Sepharose™ High Performance, Superdex™ Prep Grade and SOURCE™. The columns are also well suited for use with other Sepharose media.

When you receive your column

Please check that your column package contains the following:

- Preassembled glass tube with a protective jacket mounted between a top and a bottom assembly
- Filter kit HR 16
- Wrench
- Filter tool

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

System compatibility

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

Technical description

Column tube: High-precision borosilicate glass tube.

Protective jacket: Polyvinyl chloride (PVC) jacket which protects laboratory personnel if the column is dropped or operated outside its specifications.

Bottom assembly: A black outer bottom end-piece holding a plunger with O-ring and a preflanged tubing with an M6 tubing connector. A filter is placed between the plunger and the medium bed.

Top assembly: A red adjusting ring holding a top adaptor fitted with an O-ring. The top adaptor is connected to a preflanged tubing with an M6 tubing connector. A black top endpiece keeps the top column adaptor in a fixed position. A filter is placed between the top adaptor and the medium bed.

Bed Volumes and Heights

HR Column	With One Adapter		With Two Adapters	
	Volume (ml)	Bed Height (mm)	Volume (ml)	Bed Height (mm)
HR 16/5	5.4–13.7	27–68	0.0–13.9	0.0–69
HR 16/10	14.3–22.3	71–111	6.2–22.3	31–111
HR 16/50	95.0–103.0	475–515	87.0–103.0	335–515

First-time use

When using the column for the first time, you should dismantle, clean and reassemble the column before packing it with medium. A chromatography system should be connected for packing, packing evaluation and chromatography.

Dismantling and cleaning

To dismantle the column, proceed as follows (numbers in parentheses refer to Fig 1):

1. Unscrew the red adjusting ring (1) completely.
2. Pull the top adaptor (2) out by the red ring until it is free from the glass tube.
3. Unscrew the black top end-piece (3) from the glass tube.
4. Remove the protective jacket.
5. Unscrew the bottom end-piece (5).
6. Pull out the bottom plunger (6).
7. Rinse all parts in 20% ethanol.

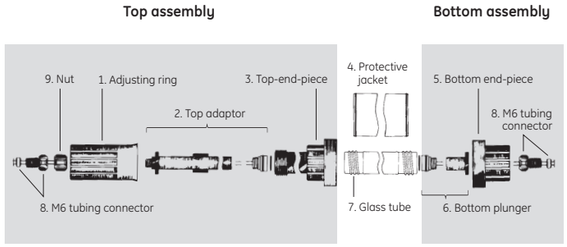

Fig 1. HR 16 column.

Assembling the column

Ensure that all parts of the column are clean. To assemble the column, proceed as follows (numbers in parentheses refer to Fig 1):

1. Wet the O-ring on the bottom plunger (6) by dipping the plunger into water, buffer or 20% ethanol. Place a wetted filter on the bottom plunger and insert it into the glass tube (7).
2. Screw the bottom end-piece (5) onto the glass tube.
3. Push the protective jacket (4) into the bottom end-piece.
4. Screw a domed nut onto the M6 tubing connector (8) attached to the outlet tubing.

Packing the column

Before starting to pack your HR 16 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 Packing Equipment HR 16, 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 an HR 16 packing connector and an HR glass tube to suit your application.

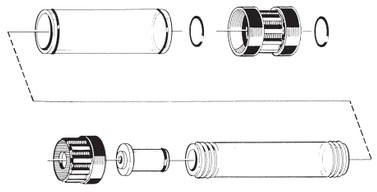

Fig 2. Packing equipment HR 16.

To pack the column, proceed as follows:

1. Assemble your column without the top assembly and attach it vertically to a stand.
2. Mount the column with the HR 16 packing equipment as described in the instructions supplied with the packing equipment. Place a beaker beneath the column tube.
3. Calculate how much chromatography medium is necessary as described in the instructions for the medium being used.
4. Pour the chromatography medium into the top of the packing tube filling both column tube and packing tube. Remove all air bubbles.
5. Connect a pump to the top of the packing unit. Remove the domed nut from the outlet tubing on the bottom assembly.
6. Pack the column by pressure or flow, depending on the instructions included with the chromatography medium.
7. When the medium is packed, switch off and disconnect the pump, refit the domed nut onto the M6 tubing connector of the outlet tubing and remove the packing tube and packing connector.
8. Top off the column with the same fluid as used for packing the column.
9. Place a filter on top of the fluid in the column and push it down to the medium surface with the wider end of the filter tool cover .
10. Rinse the walls and the top of the filter with small aliquots of buffer until all the medium particles above the filter have been removed.
11. Screw the top end-piece onto the glass tube and the protective jacket.
12. Insert the top adaptor into the top end-piece (the volume above the filter should be filled with buffer to avoid trapping air bubbles when the adaptor is inserted). Turn the adaptor unit until the two plastic guides on the adaptor line up with the tracks in the top end-piece.
13. Screw the red adjusting ring onto the threads on the top end-piece. Screw the red adjusting ring clockwise until no space is visible between the adaptor and the surface of the medium bed. Always make sure that the top inlet tubing is disconnected from the pump before adjusting the adaptor.
14. Reconnect the inlet tubing of the top assembly to the pump and remove the domed nut from the outlet tubing on the bottom assembly.
15. Start the pump and pack for 30 minutes with the same pressure or flow as in step 6 above. Mark the level of the medium in the column with a marking pen.
16. Stop the pump and disconnect the inlet tubing from the pump and refit the domed nut onto the outlet tubing.
17. Adjust the top adaptor down to the mark by rotating the red adjusting ring clockwise.
18. The column is now ready for use or storage.

Note: Do not tighten the nut (9) with a wrench. Tighten by hand only, to avoid cracking the adaptor.

Connecting the column to a system

To connect your HR 16 column to a system, proceed as follows:

1. Mount the column in a vertical position with the top assembly uppermost.
2. Ensure the inlet capillary tubing from the system as well as the preflanged inlet tubing on the column contain fluid. The system should deliver a flow of about 0.5 ml/min while the column is being connected.
3. Remove the domed nut from the inlet tubing.
4. Connect the inlet tubing to the system. For the FPLC System, the preflanged inlet tubing on the column can be fitted directly to the system. For ÄKTAsystems, a 1/16 inch male adaptor is necessary (see ordering information).
5. Immediately after connecting the inlet tubing, remove the domed nut from the column outlet tubing.
6. Wash and equilibrate the column as required.

For further information, please refer to ordering information and Amersham Biosciences BioDirectory, as well as the instrument manuals for the system being used.

Chemical resistance

The HR 16 column can be used in aqueous solutions and nearly all organic solutions commonly used in chromatography, with the following exceptions: hydrocarbons, aromatic solvents and chlorinated hydrocarbons.

Under operating conditions, the following materials are in contact with the eluent: ETFE (top and bottom assemblies, tubings), borosilicate glass, PP (filters, tubing connectors) and EPDM (O-rings).

Maintenance

Cleaning the column and parts

1. Dismantle the column (see Dismantling and cleaning above).
2. The column and its constituent parts can be cleaned in solutions of laboratory detergents. Enzyme detergents are recommended for removing contaminating proteins.
3. Wash the parts thoroughly in distilled water.
4. Reassemble the column (see Assembling the column above).

Note: With the exception of the protective jacket, components of the disassembled column may be autoclaved for 10 minutes at 120° C. Always disassemble the column before autoclaving since the differential expansion of the materials can cause physical damage.

Replacing the top filter

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 backpressure, 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. Mark the level of the medium in the column with a marking pen.
2. Start the pump and pump buffer solution, e.g. the buffer previously used, through the column at a flow rate of 1–1.5 ml/min. (Do not exceed recommended maximum backpressure.)
3. Gradually rotate the red adjusting ring counterclockwise. Unscrew the red ring completely, and stop the pump.
4. Disconnect the column top inlet tubing from the valve. Pull the adaptor out by the red ring. Unscrew the black top end-piece.
5. Check that some buffer still covers the medium bed.
6. Carefully and slowly bring up the loose filter by using the hooked filter tool. Do not press or puncture the medium bed.

Note: Only a very small amount of medium should be in suspension after removing the filter.

7. Check that the surface of the medium bed is horizontal and even. If not, use the wider end of the filter tool cover and carefully stir up 2–3 mm of medium by rotating it on the very top of the medium bed.
8. Wet a new filter in 20% ethanol. Put the filter into the column, avoiding trapping air bubbles under it. Push it gently down to the medium surface with the wider end of the filter tool cover.
9. Make sure the filter is horizontal.
10. Remove the liquid and traces of medium above the filter with a Pasteur-pipette. Rinse the walls and the top of the filter with small aliquots of buffer until all the medium particles above the filter have been removed.
11. Fill the space above the filter with buffer. Screw the top end-piece onto the glass tube and the protective jacket.
12. Check the O-ring in the adaptor. Change it if it is damaged or asymmetrical (see Replacing O-rings below).
13. Insert the adaptor, aligning it so that it engages the slots in the black top end-piece.
14. Adjust the adaptor to the pen mark by clockwise rotation of the red adjusting ring. Check that no large air bubbles have been trapped between the medium and the adaptor. If a large air bubble has become trapped, carefully remove and reposition the adaptor.
15. Reconnect the column top inlet to the valve.
16. Start the pump and pump buffer at a flow rate of 1–1.5 ml/min for 5–10 minutes to pack the top of the medium bed.
17. Stop the pump and disconnect the column inlet from the valve.
18. For final positioning of the adaptor, screw the red adjusting ring clockwise to the pen mark again.
19. The column is now ready for use. If the column is not to be used immediately, screw the domed nuts onto the M6 tubing connectors.

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, proceed as follows:

1. Disconnect the column from the system.
2. Remove the top adaptor and bring up the loose filter using the hooked filter tool.
3. Invert the column and attach to a stand over a flask that will contain the expelled medium.
4. Connect the outlet tubing of the bottom assembly to a pump.
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. Place a new filter on top of the plunger in the bottom assembly.
8. Reassemble and repack the column as described previously.

Replacing O-rings

There are O-rings in both the top and bottom assemblies.

Change the O-rings if they are damaged or asymmetrical.

Before replacing an O-ring, make sure that you have a replacement O-ring of the correct size and type.

Remove the old O-rings using forceps but take care not to damage the part on which the O-ring is fitted. Moisten a new O-ring with water to aid fitting.

Technical specifications

Materials in contact with eluent	Borosilicate glass, ETFE, PP and EPDM*
Column dimensions (inner diameter × length), mm	16 × 50 16 × 100 16 × 500
Max. operating pressure	3 MPa (30 bar, 430 psi)
Temperature, operating	+4 to +40 °C

* ETFE – Ethylene tetrafluoroethylene
PP – Polypropylene
EPDM – Ethylene propylene diene monomer

Ordering information

Columns

Product	Quantity	Code No.
Column HR 16/5	1	18-1000-98
Column HR 16/10	1	18-7403-01
Column HR 16/50	1	18-1460-01

Accessories

Product	Quantity	Code No.
Top assembly HR 16	1	18-1544-01
Bottom assembly HR 16	1	18-1545-01
Filter kit HR 16*	1	18-3585-01
Filter tool	1	18-1153-20
Packing equipment HR 16	1	18-1442-01
Packing connector HR16/10	1	18-1478-01
Packing connector HR16/16	1	18-1479-01
Chromatographic tube HR 16/10	1	18-3132-01
Chromatographic tube HR 16/50	1	18-1483-01
Union 1/16" male/M6 female	8	18-1112-58

* HR 16 Filter kit includes 10 filters and 4 O-rings

Product	Quantity	Code No.
Column Packing – The Movie	1	18-1165-33

CD-ROM containing instructional movies that demonstrate good packing techniques for gel filtration and adsorptive chromatography columns. It also shows how to check the efficiency of the column packing.

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