#### Instruction 28-9674-70 AH

## HiScale™ columns (16, 26, 50) and accessories

#### Intended use

HiScale empty columns are developed for standard liquid chromatography, optimized for process development and preparative protein purification. The columns are designed to withstand a pressure of 20 bar (2.0 MPa) and high flow rates.

## **Dimensions**

HiScale columns are available in the following dimensions:

Table 1. HiScale column dimensions

Inner diameter	Length		
(mm)	20 cm	40 cm	
16	HiScale 16/20	HiScale 16/40	
26	HiScale 26/20	HiScale 26/40	
50	HiScale 50/20	HiScale 50/40	

HiScale columns are delivered complete with a column tube and two adapters with tubing at both ends for connection to valves, pumps or monitors. As the columns are equipped with two adapters, a large range of bed heights can be obtained.

Table 2. Maximum bed volumes (ml) and bed heights (cm)

Column	Max volume (ml)	Bed heights (cm)
HiScale 16/20	40	0–20
HiScale 16/40	80	8-40
HiScale 26/20	106	0–20
HiScale 26/40	212	13-40
HiScale 50/20	393	0–20
HiScale 50/40	785	14-40

## Opening the box

Carefully take the HiScale column or adapter out of the box and check the contents against the packing list supplied. Inspect for any missing components or damage that may have occurred during transportation. Report any damage immediately to the local GE Healthcare representative and to the transport company concerned.

## **Description of main parts**

The main parts of the column are shown in Figure 1. For a detailed view of the column parts, see the exploded view on page 7.

**Glass tube** (7). The borosilicate glass tube has an inner diameter (i.d.) of  $16\pm0.02$  mm,  $26\pm0.10$  mm and  $50\pm0.50$  mm. The maximum packed bed height is 200 or 400 mm.

**Protection tube** (8). The polycarbonate plastic tube protects against splinter if the glass tube accidentally should be exposed to too high pressure and break. Together with the tube holder, the protection tube also stabilizes the column construction.

**Tube holder** (5). The red tube holder holds the column tube in position. The tube holder houses a rubber sealing and a plastic ring and it has two tabs to prevent the column from rolling when lying down.

**Adapter** (1). The adapter consists of an end cap (3), end housing (4) and a plunger (6) that carries the 20 µm net ring and support screen. The adapter has a level-adjusting mechanism that allows the plunger to be set in position in the glass tube. By turning the end knob (2), the O-ring seals against the column wall. An anti-rotator plate prevents the plunger from rotating.

**Tubing and connections**. HiScale columns are supplied with 1/16" (HiScale 16 and 26) or 1/8" (HiScale 50) tubing and fingertight connections. The tubing has an i.d. of 1.0 mm (HiScale 16 and 26) or 2.0 mm (HiScale 50) and the adapter is delivered with a union and a stop plug connected at the end of the tubing.

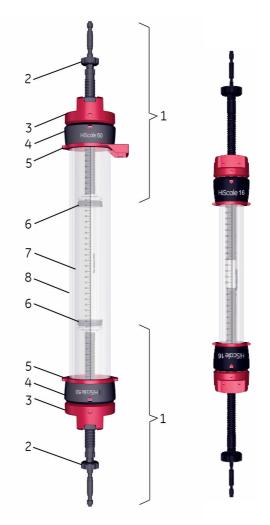


Fig 1. Main parts of the HiScale 50 (left) and HiScale 16 (right) columns.



#### Material

Under normal operating conditions, the only materials in contact with the liquid phase are: polypropylene, borosilicate glass, polyamide, fluoro-rubber, PEEK capillary tubing and ETFE ferrules.

## Chemical resistance

The columns are designed to be used in aqueous solutions and nearly all organic solvents commonly used in liquid chromatography of biomolecules, with the following few exceptions: chlorinated hydrocarbons, acetone and other ketones, aliphatic esters and phenol. However, Solutions containing more than 2 M NaOH, 1 M HCl, other strong mineral acids or 75% acetic acid should not be used. The column may be used at temperatures from 4°C to 40°C and at pressures up to 20 bar (2.0 MPa).

## Cleaning

Suitable cleaning agents are soapy water or laboratory detergents. Enzyme detergents are recommended for removing proteinaceous contaminants.

Always finish the cleaning procedure by thoroughly rinsing the column with distilled water.

#### Instructions

**Note:** Always loosen the black end knob to slacken the O-ring before inserting or removing the adapters into/from the column.

**Note:** When removing the adapters from the column, hold the tube holder (HiScale 50) in position to make sure it does not come loose.

## Dismantling the adapter

See Figure 2 for details.

- 1 Loosen the black end knob.
- 2 Unscrew the plunger.
- 3 Remove the O-ring.
- 4 Dismantle the net ring and support screen as described in "Replacing the net ring and support screen"



Fig 2. Dismantling the adapter, steps 1-2.

### Replacing the net ring and support screen

See Figure 3 for details.

- Remove the net ring (1) and support screen (2) from the plunger (3).
- 2 Put a new support screen and net ring in place.
- 3 Press on the net ring.

**Note:** To facilitate application, the net ring can be warmed in hot water (50°C to 60°C) prior to mounting.



Fig 3. Plunger with support screen and net ring

## Exchanging the capillary tubing in the adapter

See Figure 4 for details.

- Dismantle the adapter as described in "Dismantling the adapter".
- 2 Pass the tubing through the adapter inner shaft.

**Note:** When replacing the capillary tubing, it is important to use correct tubing lengths. Use a 291 mm piece of tubing for the 16 and 26 mm adapters and a 295 mm piece of tubing (available pre-cut as spare part 28-9666-56) for the 50 mm adapter.

- 3 Put on a new ferrule and screw the plunger onto the inner shaft.
- 4 Tighten the plunger firmly.
- 5 Follow the instructions in "Reassembling the adapter (HiScale 50)" and "Reassembling the adapter (HiScale 16 and HiScale 26)".



Fig 4. Exchanging the capillary tubing, steps 2-4.

#### Reassembling the adapter (HiScale 50)

See Figure 5 for details.

- 1 Connect the capillary tubing to the plunger as described in "Exchanging the capillary tubing in the adapter".
- 2 Make sure that the O-ring (1) is in position on the plunger. Slide on the expander (2) and outer shaft (3).
- 3 Slide on the anti-rotator plate (4), end housing (5), end cap (6) and assemble the end knob (7).
- 4 Put on the connector (8) and a ferrule (9) and tighten the union (10) and the stop plug (11).
- 5 The adapter is now ready for use.



Fig 5. Reassembling the adapter.

## Reassembling the adapter (HiScale 16 and HiScale 26)

See Figure 6 for details.

- 1 Connect the capillary tubing to the plunger as described in "Exchanging the capillary tubing in the adapter".
- 2 Make sure that the O-ring (1) is in position on the plunger. Slide on the outer shaft (2).
- 3 Slide on the anti-rotator plate (3), end housing (4), end cap (5) and assemble the end knob (6).
- 4 To assemble the end of the capillary tubing (7), slide on the connector (8) and tighten the connector end to the inner shaft.

**Note:** The length of tubing outside the connector should be 1 to 2 mm.

- 5 Screw the union (9) and the stop plug (10) onto the connector (8).
- 6 The adapter is now ready for use.



Fig 6. Reassembling the adapter.

# Inserting and adjusting the adapter used as bottom end piece

- 1 Insert the bottom adapter in the column tube and adjust it to the desired level.
- 2 Tighten the end knob to seal the O-ring against the column wall.

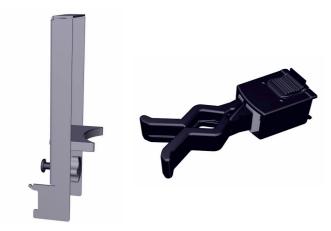
#### Packing the column

- 1 Remove the top adapter and make sure the bottom adapter is in the right place with the O-ring tightened.
- 2 Pour a small amount of packing liquid into the column tube and let it drain until the level of liquid in the tube is 2 to 3 mm above the bottom net ring.
- 3 Close the column outlet on the bottom adapter using a stop plug.
- 4 Carefully fill chromatography medium into the column tube avoiding introducing air bubbles.

- 5 Slacken the O-ring of the top adapter and insert the adapter in the column tube at an angle so that no air is trapped under the net (Fig. 7).
- 6 Fasten the adapter to the column tube by turning the end housing down.
- 7 Tighten the end knob to seal the O-ring against the column wall.
- 8 Slide the plunger slowly down a few millimeters by turning the end cap so that the air under the net ring and capillary tubing is displaced by the eluent.
- 9 Pack the column according to appropriate media instruction. If no packing method is available for HiScale, available packing methods for the XK columns should be used. For convenient handling, use a column holder to attach the column to the instrument (Fig. 8).



Fig 7. Inserting and fastening the adapter into the column tube, steps 5-6



**Fig 8.** Column holder for HiScale 50 (left) and HiScale 16 and 26 (right) compatible with ÄKTA™ systems.

### Using a packing tube

See Figure 9 for details.

When packing high beds, a separate packing tube can be used.

- 1 Attach the packing tube to the column.
- 2 Make sure that the bottom adapter is inserted and proceed packing the column as described in "Packing the column".
- 3 When the chromatography medium has settled, remove the packing tube.

**Note:** When removing the packing tube, hold the tube holder in position to make sure it does not come loose.

4 Insert the top adapter and continue packing according to appropriate media instruction.



Fig 9. Using a packing tube, step 1

## Adjusting the adapter position using QuickLock

See Figure 10 and for details.

To readjust the position of the adapter in the column:

- 1 Stop the pump.
- 2 Close the column outlet using a stop plug.
- 3 Disconnect the column from the pump leaving the inlet tubing open.
- 4 Loosen the end knob of the top adapter to slacken the O-ring.
- 5 Press in the QuickLock and adjust the adapter to a position just above the bed level.
- 6 Tighten the end knob to obtain a good seal.
- 7 Fine-tune the adapter position by turning the end cap.
- 8 Turning of the end cap can also be used for axial compression of the gel bed as described in the media instruction. If needed, use a spanner to turn the end cap. To prevent the column from rotating, a second spanner to hold the end housing can be used.

9 After the column is packed, the chamber above the adapter can be rinsed by injecting liquid through the holes in the end housing.



Fig 10. Adjusting the adapter position using QuickLock, steps 4-5.

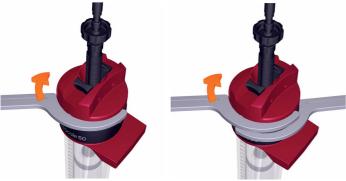


Fig 11. Using spanner(s) to turn the end cap, step 8.

# Ordering information

Column*	Article no.	
HiScale 16/20	28-9644-41	
HiScale 16/40	28-9644-24	
HiScale 26/20	28-9645-14	
HiScale 26/40	28-9645-13	
HiScale 50/20	28-9644-45	
HiScale 50/40	28-9644-44	

<sup>\*</sup> Each HiScale column is delivered with two adapters

## **Accessories**

	HiScale 16	HiScale 26	HiScale 50	No. per pack
Spanner wrench	28-9647-76	28-9647-77	28-9647-78	2
Long column holder	18-1126-32	18-1126-32		1
Short column holder	18-1113-17	18-1113-17		1
Column holder ÄKTA avant	28-9562-82	28-9562-82		1
Column clamp ÄKTA avant	28-9563-19	28-9563-19		1
Column holder, steel			28-9644-99	1
Superloop, 1/16" fittings 10 ml	18-1113-81	18-1113-81	18-1113-81	1
Superloop, 1/16" fittings 50 ml	18-1113-82	18-1113-82	18-1113-82	1
Superloop, M6 fittings 150 ml	18-1023-85	18-1023-85	18-1023-85	1
Union 5/16" female - 1/16" male			18-1142-08	8
Fingertight union 1/16" male/M6 female	18-1112-58	18-1112-58	18-1112-58	8
Connector 1/16" male/Luer female	18-1112-51	18-1112-51		2
Net ring 10 µm	18-8761-01	18-8760-01	18-8759-01	5
Net ring 80 µm			18-1000-69	5
Tubing 1 mm i.d.	18-1115-83	18-1115-83		2 m
Tubing 2 mm i.d.			28-9663-76	1 m
Tubing cutter	18-1112-46	18-1112-46		1
Column tube 20	28-9666-46	28-9666-48	28-9666-49	1
Column tube 40	28-9666-52	28-9666-51	28-9666-50	1
Packing tube 20	28-9868-16	28-9803-83	28-9802-51	1
Packing tube 40	28-9868-15	28-9645-05	28-9645-06	1
O-ring, packing tube	28-9666-53	28-9666-54	28-9666-55	2
Accessory kit*	28-9663-67	28-9663-74	28-9663-75	1

 $<sup>\</sup>mbox{\ensuremath{^{\star}}}$  The accessory kit includes 1 net ring, 1 support screen, 1 O-ring,

## Spare parts

The exploded views (Fig. 12 and Fig. 13) shows the positions of the different parts of the HiScale columns. For replacements, please order according to the spare parts list using the appropriate article numbers.

Item no.	Article no. designation	HiScale 16	HiScale 26	HiScale 50	Material	No. per pack
1	Adapter	28-9663-83	28-9663-84	28-9663-85		1
8	Column tube 20	28-9666-46	28-9666-48	28-9666-49	A, B, C, D, E	1
8	Column tube 40	28-9666-52	28-9666-51	28-9666-50	A, B, C, D, E	1
9	Fingertight stop plug, 1/16"	11-0003-55	11-0003-55		F	5
9	Stop plug 5/16" male			18-1112-50	F	5
10	Union Valco F/F	11-0003-39	11-0003-39		F	5
10	Connector 5/16" female - 5/16" female			18-1173-51	F	2
20	Ferrules for 1/16" tubing	18-1127-06	18-1127-06		G	10
11,20	Ferrules for 1/8" tubing			18-1121-18	G	10
12	Connector for 1/16" tubing	29-0183-82	29-0183-82		F	2
12	Connector 1/8"			18-1121-17	F	10
17	Tubing 2 mm i.d. cut			28-9666-56	F	0.295 m
23	O-ring	19-0163-01	28-9782-27	28-9782-28	Н	5
25	Support screen	19-0651-01	18-9377-01	19-0664-01	1	5
26	Net ring 20 µm	28-9663-79	28-9663-80	28-9663-81	I, J	5

## Material

A =	Borosilicate glass
B =	Polycarbonate
C =	Polyamide (reinforced glass fibre)
D =	Ethylene propylene diene polymer (EPDM)
E =	Polyoxymetylen (POM)
F =	Polyether ether ketone (PEEK)
G =	Ethylene tetrafluoroethylene (ETFE)
H =	Fluoro-rubber (Viton™)
l =	Polypropylene
J =	Polyamide (nylon)

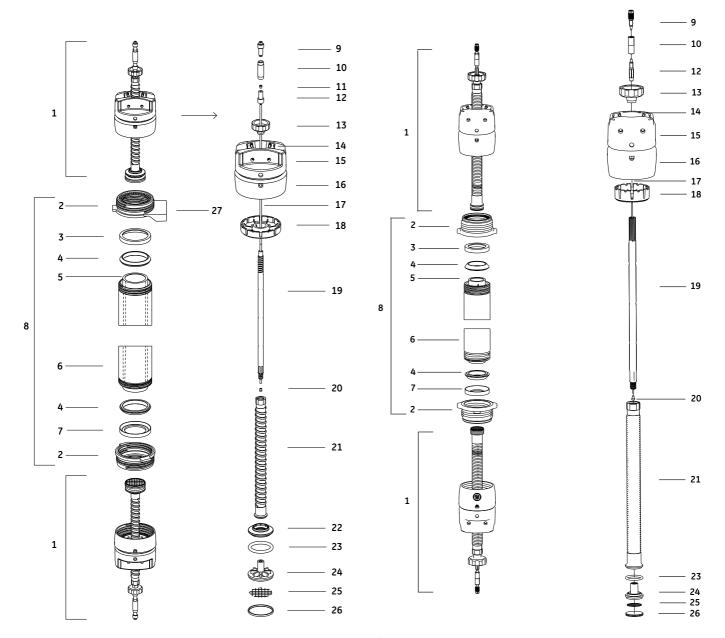


Fig 12. Exploded view of the HiScale 50 column

Fig 13. Exploded view of the HiScale 16 and 26 columns

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