

HiScale[™] columns (10, 16, 26, 50) and accessories Instructions for Use

Intended use

HiScale[™] empty columns are developed for standard liquid chromatography, and optimized for process development, scaledown studies, as well as preparative protein purification. The columns are designed to withstand a pressure of 2.0 MPa (20 bar) 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	
10		HiScale 10/40	
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 with a column tube and two adjustable adapters. As the columns are equipped with an adapter at both ends, a large range of bed heights can be obtained, see the table below.

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

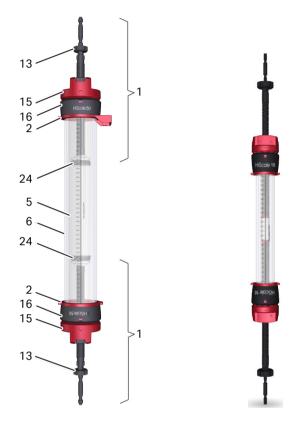
Column	Maximum bed volume (mL)	Bed height (cm)
HiScale 10/40	31	10-40
HiScale 16/20	40	0–20
HiScale 16/40	80	8-40
HiScale 26/20	106	0-20
HiScale 26/40	212	13–40
HiScale 50/20	393	0–20
HiScale 50/40	785	14–40

Unpacking

Unpack the HiScale column and the adapters. Use the packing list supplied to make sure that no components are missing. Examine the components for transport damage. Report any damage immediately to the local Cytiva representative and to the transport company concerned.

Description of main parts

The main parts of the column are shown in *Fig. 1, on page 1*. For a detailed view of the column parts, see the exploded views in *Exploded views of the columns, on page 8*.



Note: Numbering corresponds to the exploded views in Exploded views of the columns, on page 8.

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

Glass tube (5). The borosilicate glass tube has an inner diameter (i.d.) of 10 mm, 16 mm, 26 mm, or 50 mm. The maximum packed bed height is 200 mm or 400 mm.

Protection tube (6). The polycarbonate plastic tube protects against splinter if the glass tube breaks because of excessive pressure. The protection tube and the tube holder also stabilize the column construction.

Tube holder (2). 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 laid down.

Adapter (1). The adapter consists of an end cap (15), an end housing (16), and a plunger (24) that carries the 20 µm net ring and the support screen. The adapter has a level-adjusting mechanism that allows the plunger to be positioned in the glass tube. The O-ring seals against the column wall by turning the end knob (13). An anti-rotator plate prevents the plunger from rotating.

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Tubing and connections. HiScale columns are supplied with internal tubing preassembled, see the table below for the dimensions. At the top of the HiScale 16, 26, and 50 adapters, a union and a stop plug are connected to the internal tubing end. At the top of the HiScale 10 adapters, however, no union and no stop plug are connected, and the internal tubing continues externally. Fingertight connectors for the HiScale 10 column are found in the accessory kit included in the package.

Table 3. Dimensions of tubing and fingertight connectors supplied.

Column	Tut	Tubing		
Column	i.d.	o.d.	connectors	
HiScale 10	0.75 mm	1/16″	1/16″	
HiScale 16	1 mm	1/16″	1/16″	
HiScale 26	1 mm	1/16″	1/16″	
HiScale 50	2 mm	1/8″	5/16″	

Materials

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

Chemical resistance

The columns are designed for use with aqueous solutions and most organic solvents commonly used in liquid chromatography of biomolecules, except for: chlorinated hydrocarbons, acetone and other ketones, aliphatic esters, and phenol. Solutions containing more than 2 M NaOH, 1 M HCl, other strong mineral acids, or 75% acetic acid must not be used either. The columns can be used at temperatures from 4°C to 40°C and at pressures up to 2.0 MPa (20 bar).

Cleaning

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

Always finish the cleaning procedure by rinsing the column thoroughly 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 that it does not come loose.
- **Note:** Before each column packing, make sure that the ferrule bottom and capillary tubing end are aligned, and that the plunger is tightened according to Fig. 4, on page 3 and Fig. 5, on page 3 (HiScale 10) to avoid leakage.

It is also important to perform a sharp and straight cut of the capillary tubing, see *Fig. 13, on page 5*.

Disassemble the adapter

See Fig. 2, on page 2 for details.

Step Action

1

2

3

Δ

5 6

For HiScale 16, 26, and 50 only: Unscrew and remove:

- a. the stop plug (9),
- b. the union (10), and
- c. the connector (12).
- Unscrew the black end knob (13).
- Slide the inner shaft (19) out from the outer shaft (21).
- Unscrew the plunger (24).
- Remove the O-ring (23).
- Disassemble the net ring and the support screen as described in *Replace the net ring and the support screen, on page 2.*



* 9, 10 and 12 are not included in the HiScale 10 design.

Fig 2. Disassembling the adapter.

Replace the net ring and the support screen

See Fig. 3, on page 2 for details.

Step Action

- 1 Remove the net ring (26) and the support screen (25) from the plunger (24).
- 2 Place a new support screen and a new net ring on the plunger.
- 3 Press on the net ring.

Note:

The net ring is easier to press on after heating it in hot water (50°C to 60°C).



Fig 3. Plunger with support screen and net ring.

* Not included in HiScale 10 design.

Replace the capillary tubing in the adapter

See Fig. 4, on page 3 for details.

Use the correct tubing length, when replacing the capillary tubing. The HiScale 50 tubing is available pre-cut as spare part 28966656.

Adapter	Tubing length required	
HiScale 10	700 mm	
HiScale 16	201 mm	
HiScale 26	- 291 mm	
HiScale 50	295 mm	

Step Action

- 1 Disassemble the adapter as described in *Disassemble the* adapter, on page 2.
- 2 Pass the tubing through the adapter inner shaft.
- 3 Place a new ferrule and make sure that the capillary tubing end and the ferrule bottom are aligned. Screw the plunger onto the inner shaft.
- 4 Tighten the plunger firmly according to Figure 4 and Figure 5 below (for HiScale 10).
- 5 Follow the instructions in *Reassemble the adapter (HiScale 10, 16, and 26), on page 3* and *Reassemble the adapter (HiScale 50), on page 4.*



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

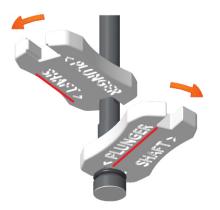


Fig 5. For HiScale 10: The plunger is to be finger-tightened to the inner shaft. However, if a proper seal at the ferrule is not achieved, the wrenches can be used. Place the designated end of the wrench (< PLUNGER or SHAFT >) over the flats on the corresponding item to grip it. Move the wrenches carefully, no more than half a turn of the plunger from its finger-tightened position in order not to break the plunger.

Reassemble the adapter (HiScale 10, 16, and 26)

See Fig. 6, on page 3 for details.

Step Action

1

2

3

- Connect the capillary tubing to the plunger as described in *Replace the capillary tubing in the adapter, on page 3*.
- Make sure that the O-ring (23) is in position on the plunger (24). Slide on the outer shaft (21).
- Slide on the anti-rotator plate (18), the end housing (16), the end cap (15), and assemble the end knob (13) gently without forcing the components into position.
- 4 To assemble the end of the capillary tubing (17), slide on the connector (12), and tighten the connector end to the inner shaft (19).

Note:

Make sure the connector and the inner shaft designs are compatible with each other. See Connector compatibility, on page 7 for details.

Note:

The length of the tubing outside of the connector must be 1 to 2 mm.

- 5
- For HiScale 16 and 26 only: Screw the union (10) and the stop plug (9) onto the connector (12).

The adapter is now ready for use.



* 9, 10, and 12 are not included in HiScale 10 design.

Fig 6. Reassembling the adapter.

Reassemble the adapter (HiScale 50)

See Fig. 7, on page 4 for details.

Step Action

1	Connect the capillary tubing to the plunger as described in
	Replace the capillary tubing in the adapter, on page 3.

- 2 Make sure that the O-ring (23) is in position on the plunger. Slide the expander (22) and the outer shaft (21) onto the tubing.
- 3 Slide on the anti-rotator plate (18), the end housing (16), the end cap (15), and assemble the end knob (13).
- 4 Attach a connector (12) and a ferrule (11), and tighten the union (10) and the stop plug (9).

The adapter is now ready for use.



Fig 7. Reassembling the adapter.

Insert and adjust the adapter used as a bottom end piece

Step Action

- 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.

Note:

Before each column packing, make sure that the ferrule is compressed thoroughly by tightening the plunger to the inner shaft, see Fig. 5, on page 3.

Pack the column

Refer to the Instructions for Use for the respective resin for the slurry concentration and other parameters.

Step Action

- 1 Remove the top adapter and make sure that the bottom adapter is in the right position 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.

Step Action

- 3 Close the column outlet on the bottom adapter using a stop plug.
- 4 Carefully fill chromatography resin into the column tube to avoid 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, see *Fig. 8, on page 4.*
- 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 any air under the net ring and capillary tubing is displaced by the eluent.
- 9 Pack the column according to the appropriate resin instruction. If no packing method is available for HiScale, use available packing methods for the XK columns. For convenient handling, use a column holder to attach the column to the instrument, see *Fig. 9, on page 4*.

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



Fig 8. Inserting and fastening the adapter into the column tube, steps 5-6. Step 6 Fasten the adapter to the column tube by turning the end housing down. Holding the end cap still while doing so keeps the shaft and plunger from spinning.



Fig 9. Column holder for HiScale 50 (left) and HiScale 10, 16, and 26 (right) compatible with ÄKTA™ systems.

Use a packing tube

See Fig. 10, on page 5 for details.

A separate packing tube can be used when packing high beds. The table below specifies the bed heights for the respective columns for which the use of a packing tube is recommended.

Column	Packing tube recommended for bed heights
HiScale 10/40	> 20 cm
HiScale 16/20	
HiScale 26/20	> 12 cm
HiScale 50/20	
HiScale 16/40	
HiScale 26/40	> 25 cm
HiScale 50/40	

Step Action

- 1 Attach the packing tube to the column.
- 2 Make sure that the bottom adapter is inserted and proceed with packing the column as described in *Pack the column*, *on page 4*.
- 3 When the chromatography resin has settled, remove the packing tube.

Note:

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

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

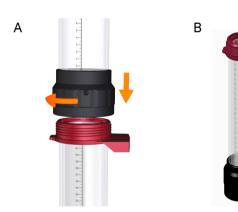


Fig 10. A: Using a packing tube, step 1. B: Packing tube.

Adjust the adapter position using QuickLock

See Fig. 11, on page 5 and Fig. 12, on page 5 for details.

Follow the steps below to adjust the position of the column adapter.

Step Action

- 1 Stop the pump.
- 2 Close the column outlet using a stop plug.
- 3 Disconnect the column from the pump and leave the inlet tubing open.
- 4 Loosen the end knob of the top adapter to slacken the O-ring.

Step Action

- 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.
- **Note:** If needed, use a spanner wrench to turn the end cap. To prevent the column from rotating, a second spanner wrench to hold the end housing can be used.



Fig 11. Adjusting the adapter position using QuickLock, steps 4–5.

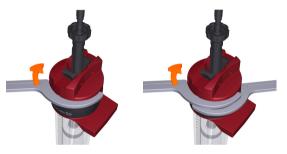


Fig 12. Using one spanner wrench, or two, to turn the end cap.

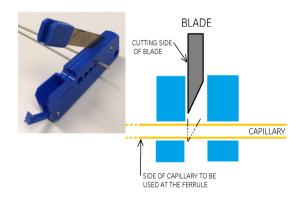


Fig 13. Equipment and instructions for cutting capillary tubing.

Ordering information

Column ¹	Product code
HiScale 10/40	29360550
HiScale 16/20	28964441
HiScale 16/40	28964424
HiScale 26/20	28964514
HiScale 26/40	28964513
HiScale 50/20	28964445
HiScale 50/40	28964444

¹ Each HiScale column is delivered with two adapters.

Accessories

	HiScale 10	HiScale 16	HiScale 26	HiScale 50	Qty per pack
Spanner wrench	28964776	28964776	28964777	28964778	2
Long column holder, ÄKTA classic	18112632	18112632	18112632		1
Short column holder, ÄKTA classic		18111317	18111317		1
Column holder ÄKTA avant™, ÄKTA pure™	28956282	28956282	28956282		1
Column holder, steel, ÄKTA avant				28964499	1
Superloop™, 1/16″ fittings 10 mL	18111381	18111381	18111381	18111381	1
Superloop, 1/16" fittings 50 mL	18111382	18111382	18111382	18111382	1
Superloop, M6 fittings 150 mL	18102385	18102385	18102385	18102385	1
Union 5/16" female - 1/16" male				18114208	8
Fingertight union 1/16" male/M6 female	18111258	18111258	18111258	18111258	8
Connector 1/16" male/Luer female	18111251	18111251	18111251		2
Net ring 10 µm	29360547	18876101	18876001	18875901	5
Net ring 80 µm				18100069	5
Tubing 0.75 mm i.d., 1/16" o.d.	18111253				2 m
Tubing 1 mm i.d., 1/16" o.d.		18111583	18111583		2 m
Tubing 2 mm i.d., 1/8" o.d.				28966376	1 m
Tubing cutter	18111246	18111246	18111246		1
Column tube 20		28966646	28966648	28966649	1
Column tube 40	29372270	28966652	28966651	28966650	1
Packing tube 20, complete	29360551	28986816	28980383	28980251	1
Packing tube 40, complete		28986815	28964505	28964506	1
O-ring, packing tube	18103579	28966653	28966654	28966655	2
Accessory kit ¹	29360581	28966367	28966374	28966375	1

¹ The accessory kit includes 1 net ring, 1 support screen (not included for HiScale 10), 1 O-ring, 2 ferrules, and 2 fingertight stop plugs.

Spare parts

The illustrations in *Exploded views of the columns, on page 8* show the positions of the different parts of the HiScale columns. For replacements, order according to the spare parts list using the appropriate product codes.

ltem no.	Product code designation	HiScale 10	HiScale 16	HiScale 26	HiScale 50	Material	Qty per pack
1	Adapter	29372404	29751037	29751038	28966385		1
8	Column tube 20		28966646	28966648	28966649	A, B, C, D, E	1
8	Column tube 40	29372270	28966652	28966651	28966650	A, B, C, D, E	1
9	Fingertight stop plug, 1/16" male		11000355	11000355		F	5
9	Stop plug 5/16" male				18111250	F	5
10	Union 1/16" female/ 1/16" female		11000339	11000339		F	5
10	Union 5/16" female - 5/16" female				18117351	F	2
20	Ferrules for 1/16" tubing	18112706	18112706	18112706		G	10
11,20	Ferrules for 1/8" tubing				18112118	G	10
12, 19	Inner shaft and connector for HiScale 16 and 26 ¹		29751036	29751036		F	2
12	Connector for 1/8" tubing				18112117	F	10
17	Tubing 2 mm i.d. cut				28966656	F	0.295 m
23	O-ring	29372271	19016301	28978227	28978228	D, H	5
25	Support screen		19065101	18937701	19066401	I	5
26	Net ring 20 µm	29372273	28966379	28966380	28966381	I, J	5
24	Distributor HiScale 10 (Plunger)	29412496				F	1
2	Wrench HiScale 10	29372274				J	2

¹ See compatibility information below for the current design.

² Not included in the exploded view. See *Fig. 5, on page 3.*

Materials

А	=	Borosilicate glass
В	=	Polycarbonate
С	=	Polyamide (reinforced glass fibre)
D	=	Ethylene propylene diene polymer (EPDM)
Е	=	Polyoxymethylene (POM)
F	=	Polyether ether ketone (PEEK)
G	=	Ethylene tetrafluoroethylene (ETFE)
Н	=	Fluoro-rubber (Viton™)
Ι	=	Polypropylene
J	=	Polyamide (nylon)

Connector compatibility

For HiScale 16 and 26 columns, the current design of the inner shaft is only compatible with the current connector. Previous designs of the connector can only be used with previous designs of the inner shaft. See the table below to identify the compatible designs.

Part	Previous design	Current design
Connector (12)		
Inner shaft (19)	260 mm length	264 mm length

Both the inner shaft and the connector must be replaced when updating to the current design.

Exploded views of the columns

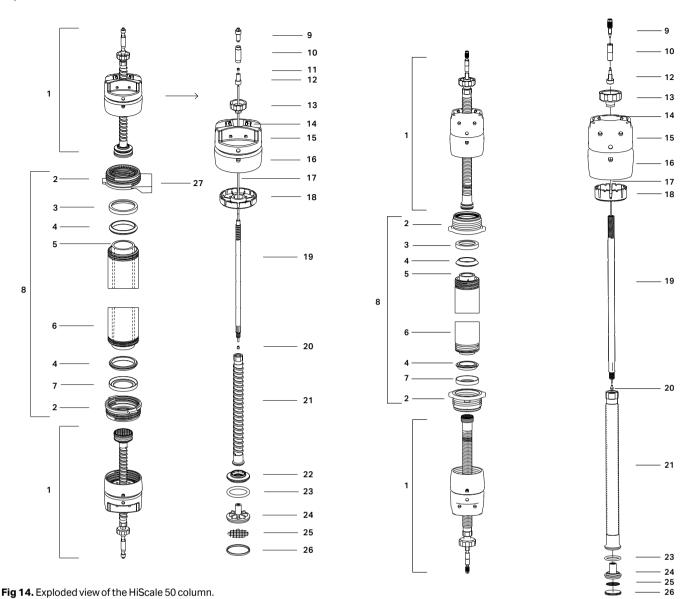
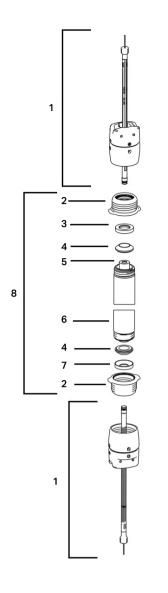
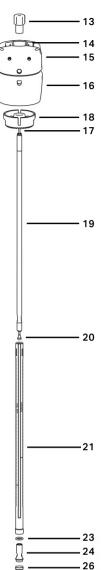


Fig 14. Exploded view of the HiScale 50 column.

Fig 15. Exploded view of the HiScale 16 and 26 columns.

1	Adapter	15	End cap
2	Tube holder	16	Endhousing
3	Sealing ring	17	PEEK tubing
4	Sealing	18	Anti-rotator plate
5	Glass tube	19	Innershaft
6	Protection tube	20	Ferrule
7	Stop ring	21	Outer shaft
8	Column tube	22	Expander (HiScale 50 only)
9	Stop plug	23	O-ring
10	Union	24	Plunger
11	Ferrule (HiScale 50 only)	25	Support screen
12	Connector (1/8" or 1/16")	26	Net ring
13	End knob	27	Hook for column holder (HiScale 50 only)
14	QuickLock		





1	Adapter
2	Tubeholder
3	Sealing ring
4	Sealing
5	Glass tube
6	Protection tube
7	Stop ring
8	Column tube
13	End knob
14	QuickLock
15	End cap
16	End housing
17	PEEK tubing
18	Anti-rotator plate
19	Innershaft
20	Ferrule
21	Outer shaft
23	O-ring
24	Plunger
26	Net ring

Fig 16. Exploded view of the HiScale 10 column.



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