

HiScale columns (10, 16, 26, 50) and accessories

Instructions

Intended use

HiScale™ empty columns are developed for standard liquid chromatography, optimized for process development, scale down studies, and 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 (mm)	Length	
	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 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 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

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 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 on [Fig. 14, on page 8](#) to [Fig. 16, on page 9](#).

Glass tube (7). The borosilicate glass tube has an inner diameter (i.d.) of 10 mm, 16 mm, 26 mm and 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" o.d. (HiScale 10, 16, and 26) or 1/8" o.d. (HiScale 50) tubing and fingertight connections - 1/16" (HiScale 16 and 26) or 5/16" (HiScale 50). For HiScale 10, the connections are available in the accessory kit included in the package. The tubing has an i.d. of 0.75 mm (HiScale 10) 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 must not be used. The column 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 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.

Note: Prior to each column packing ensure that the ferrule bottom and capillary 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 ensure a sharp and straight cut of the capillary. See Fig. 13, on page 5.

Dismantling the adapter

See Figure 2 below for details.

Step	Action
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 below for details

Step	Action
1	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.

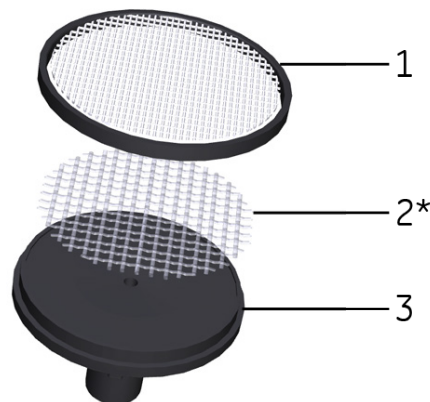


Fig 3. Plunger with support screen and net ring.

* Not included in HiScale 10 design

Exchanging the capillary tubing in the adapter

See Figure 4 below for details.

Step	Action
1	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 700 mm piece of tubing for HiScale 10 adapters, 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 28966656) for the 50 mm adapter.

- | | |
|---|---|
| 3 | Put on a new ferrule and make sure that the capillary 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 "Reassembling the adapter (HiScale 50)" and "Reassembling the adapter (HiScale 10, 16 and HiScale 26)". |



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

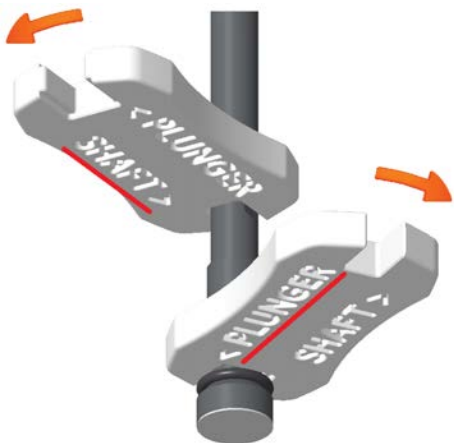


Fig 5. For HiScale 10: The plunger is to be finger-tightened to the inner shaft. However, if a proper seal at the ferrule is not achieved, the wrenches can be used. Put the designated end of the wrench ("PLUNGER" or "SHAFT") over the flats on corresponding item to grip it. Move the wrenches carefully,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

Reassembling the adapter (HiScale 50)

See Figure 6 below for details.

Step	Action
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 6. Reassembling the adapter.

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

See [Fig. 7, on page 3](#) below for details.

Step	Action
1	Connect the capillary tubing to the plunger as described in Exchanging the capillary tubing in the adapter, on page 2 .
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) gently without forcing the components into position.
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: <i>The length of tubing outside the connector must be 1 to 2 mm.</i>	
5	Screw the union (9) and the stop plug (10) onto the connector (8). (not applicable for HiScale 10)
6	The adapter is now ready for use.



* 8,9 and 10 not included in HiScale 10 design.

Fig 7. Reassembling the adapter.

Inserting and adjusting the adapter used as 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:

Prior to each column packing, make sure that the ferrule is thoroughly compressed by tightening the plunger to the inner shaft. See [Fig. 5, on page 3](#).

Packing the column

Step Action

- 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. 8 below).
- 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 resin 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. 9 below).



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 AKTA™ systems.

Using a packing tube

See [Fig. 10, on page 5](#) for details.

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

Step Action

- 1 Attach the packing tube¹ to the column.
- 2 Make sure that the bottom adapter is inserted and proceed packing the column as described in [Packing 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 it does not come loose.
- 4 Insert the top adapter and continue packing according to appropriate resin instruction.

¹ Packing tube is recommended for bed heights above 12 cm for HiScale 16/20, 26/20 and 50/20, 20 cm for HiScale 10/40 and bed heights above 25 cm for HiScale 16/40, 26/40 and 50/40.

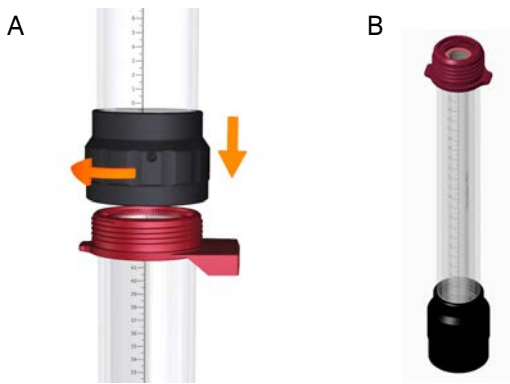


Fig 10. A: Using a packing tube, step 1. **B:** Packing tube.

Adjusting the adapter position using QuickLock

See Fig 11 and Fig 12 below for details.

To readjust the position of the adapter in the column:

Step	Action
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 11. Adjusting the adapter position using QuickLock, steps 4-5.

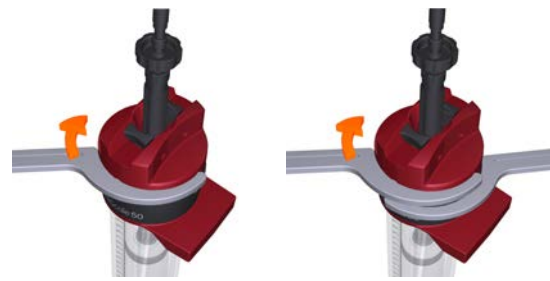


Fig 12. Using spanner(s) to turn the end cap, step 8.

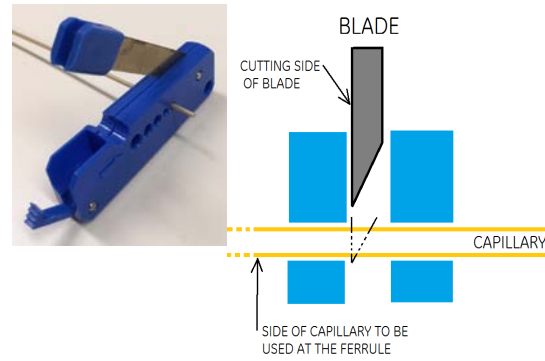


Fig 13. Equipment and instruction for cutting capillary

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	No. per pack
Spanner wrench	28964776	28964776	28964777	28964778	2
Long column holder, classic ÄKTA	18112632	18112632	18112632		1
Short column holder, classic ÄKTA		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 exploded views ([Fig. 14, on page 8](#) to [Fig. 16, on page 9](#)) shows the positions of the different parts of the HiScale columns. For replacements, order according to the spare parts list using the appropriate product codes..

Item no.	Product code designation	HiScale 10	HiScale 16	HiScale 26	HiScale 50	Material	No. per pack
1	Adapter	29372404	28966383	28966384	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				181117351	F	2
20	Ferrules for 1/16" tubing	18112706	18112706	18112706		G	10
11,20	Ferrules for 1/8" tubing				18112118	G	10
12	Connector for 1/16" tubing		29018382	29018382		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				J	1
¹	Wrench HiScale 10	29372274				J	2

¹ Not included in the exploded view. See [Fig. 5, on page 3](#).

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™)
I	=	Polypropylene
J	=	Polyamide (nylon)

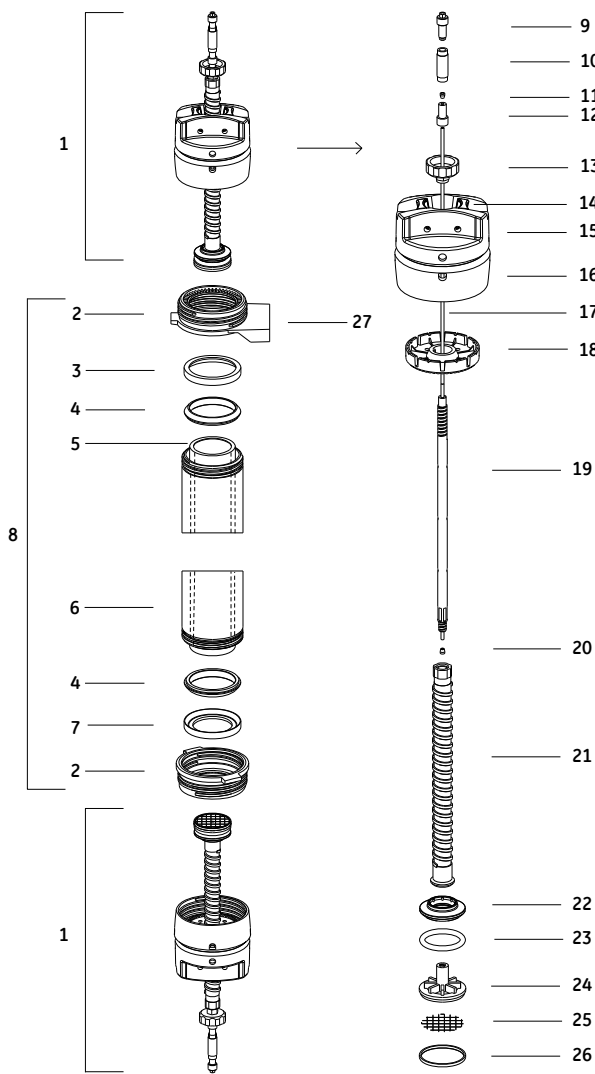


Fig 14. Exploded view of the HiScale 50 column

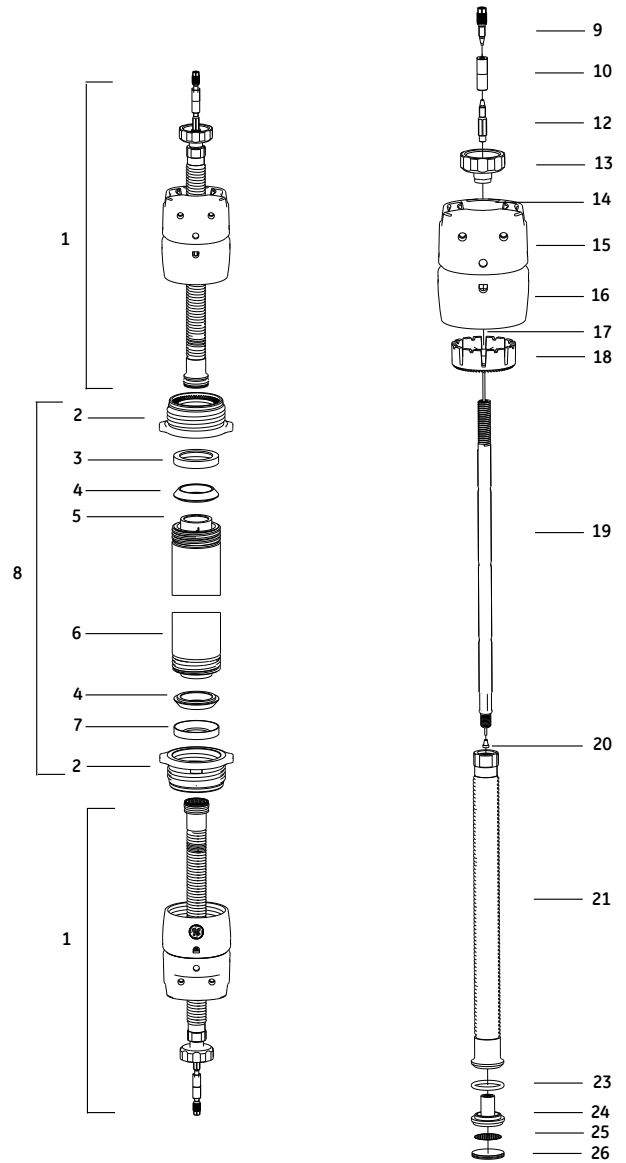


Fig 15. Exploded view of the HiScale 16 and 26 columns

1	Adapter	15	End cap
2	Tube holder	16	End housing
3	Sealing ring	17	Peek tubing
4	Sealing	18	Anti-rotator plate
5	Glass tube	19	Inner shaft
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"/ Connector for 1/16" tubing	26	Net ring
13	End knob	27	Hook for column holder (HiScale 50 only)
14	QuickLock		

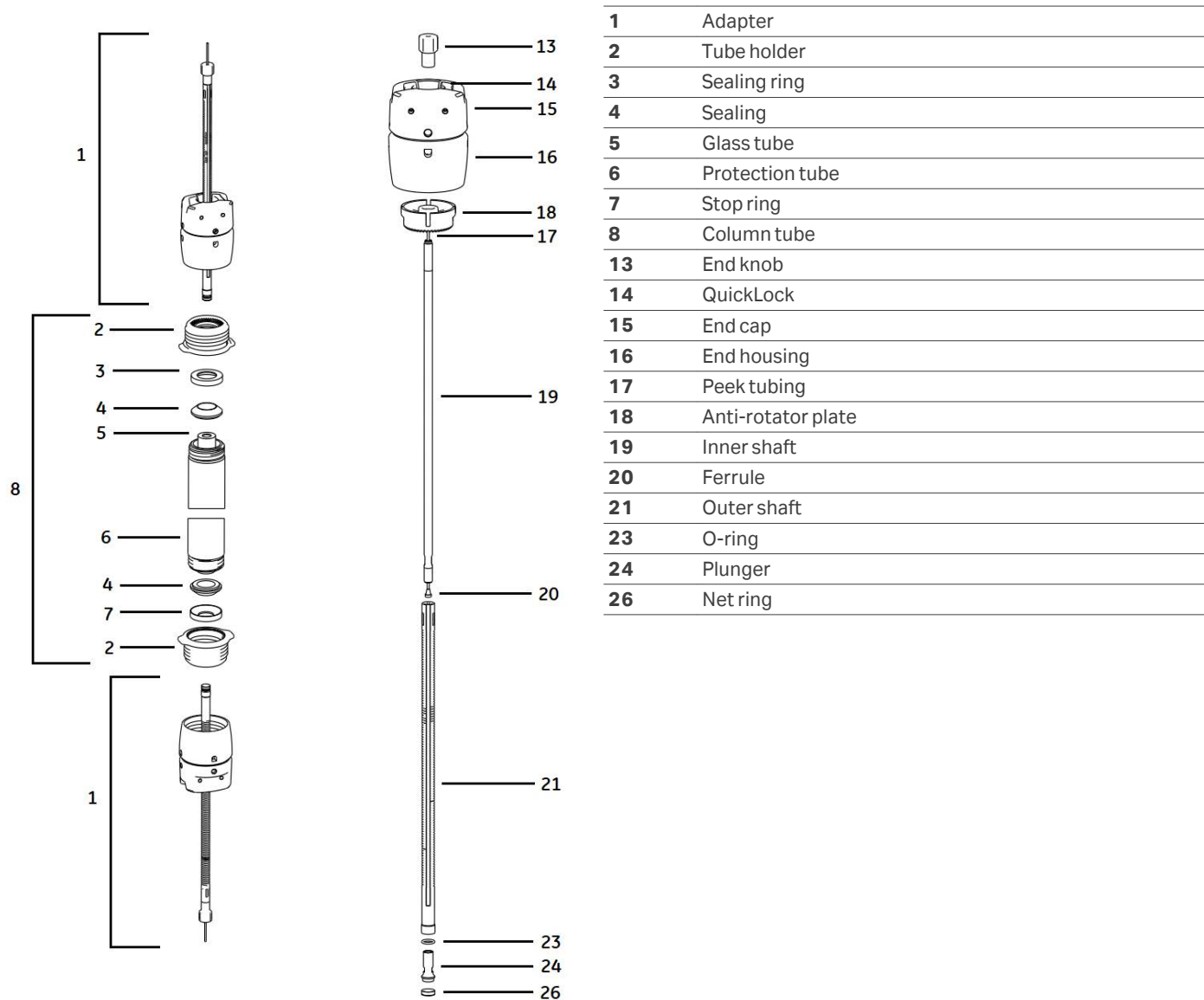


Fig 16. Exploded view of the HiScale 10 column

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