GE Healthcare
Life Sciences

Instruction 28-9920-17 AC

HiLoad™ 16/600 and 26/600 Superdex™ 30 prep grade
HiLoad 16/600 and 26/600 Superdex 75 prep grade
HiLoad 16/600 and 26/600 Superdex 200 prep grade

Introduction

HiLoad 16/600 and 26/600 Superdex 30 prep grade, HiLoad 16/600 and 26/600 Superdex 75 prep grade, and HiLoad 16/600 and 26/600 Superdex 200 prep grade (pg) are prepacked XK columns designed for preparative gel filtration.

Superdex prep grade is a composite matrix of dextran and highly cross-linked agarose. The steep selectivity of dextran and the high chemical and physical stability of agarose enable high resolution separations. Steep selectivity curves give unmatched resolution for biomolecules in the molecular weight range up to 10 000 for Superdex 30 pg, 3 000 to 70 000 for Superdex 75 pg, and 10 000 to 600 000 for Superdex 200 pg (Fig 2).

The chromatography media combines high mechanical strength with high hydrophilicity, allowing high flow rates and minimal non-specific interactions.

Table 1: Contents of the delivery box

Component	No. supplied
Transport device	1
1/16" male connectors	2
Stop plug	1
HiLoad column	1
Instructions	1

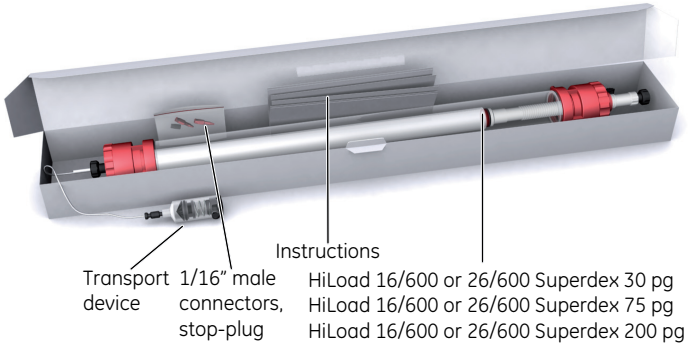


Fig. 1 Package includes HiLoad column, transport device, two connectors, two stop plugs and instructions.

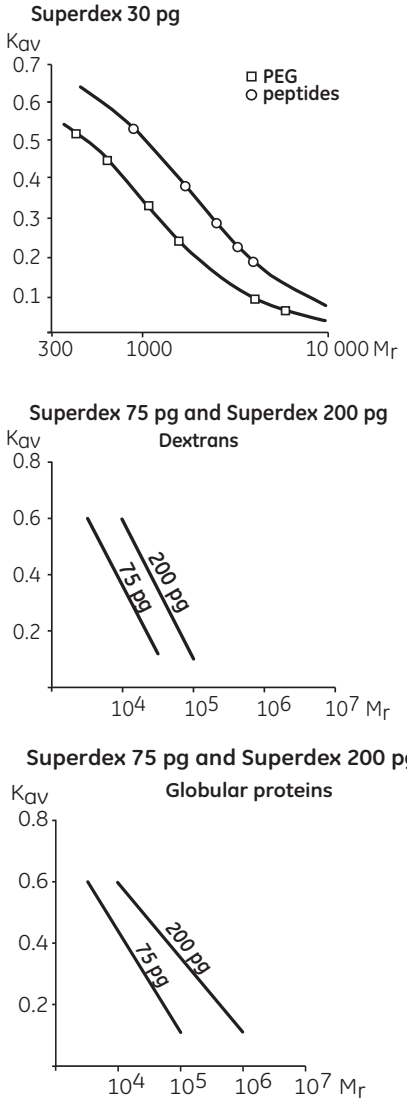


Fig. 2 Selectivity curves from Superdex 30 pg, Superdex 75 pg and Superdex 200 pg.

Table 2: HiLoad column characteristics

Matrix	Dextran covalently bound to highly cross-linked agarose
Mean particle size	34 µm
Separation range (M_r) Globular proteins Dextrans	< 10 000 (Superdex 30 pg) 3×10^3 to 7×10^4 (Superdex 75 pg) 1×10^4 to 6×10^5 (Superdex 200 pg) 5×10^2 to 3×10^4 (Superdex 75 pg) 1×10^3 to 1×10^5 (Superdex 200 pg)
Column volume*	120 to 124 ml (16/600) 319 to 330 ml (26/600)
Sample volume†	Up to 5 ml (16/600) Up to 13 ml (26/600)
Recommended flow rate	30 cm/h at room temperature (1 ml/min for 16/600 or 2.6 ml/min for 26/600)
Recommended flow rate range	10 to 50 cm/h at room temperature (0.3 to 1.6 ml/min for 16/600 or 0.9 to 4.4 ml/min for 26/600)
Theoretical plates	> 13 000 m^{-1}
Maximum pressure over the packed bed during operation, Δp	0.3 MPa, 3 bar, 42 psi
Column hardware pressure limit‡	0.5 MPa, 5 bar, 73 psi
pH stability: long term and working range short term	 3 to 12 1 to 14
Storage: - Superdex 30 - Superdex 75 - Superdex 200	 - 0.2 M sodium acetate, 20% ethanol at room temperature - 0.2 M sodium acetate, 20% ethanol at room temperature - 20% ethanol at room temperature

* The surface of the medium is not directly visible at the bottom piece. Therefore, when calculating the total column volume, calculate the height of the medium from the lowest part of the bottom piece to the surface of the medium/adapter. For HiLoad 16/600 deduct 30 mm, and for HiLoad 26/600 deduct 36 mm.

† Optimal sample volume depends on the complexity of the sample and the flow rate. If the sample contains substances with small differences in size, either decrease the sample volume, or decrease the flow rate (in very difficult cases, it may be necessary to decrease both).

‡ See "Adjusting pressure limits in chromatography system software".

First time use

Connecting the column

1 Before connecting the column to a chromatography system, start the pump to remove any air bubbles from the system, particularly in the tubing and valves.

2 Stop the pump.

3 Mount the column vertically, remove the stop plug and connect the inlet tubing to the system "drop-to-drop".

4 Remove the transport device and connect the column outlet tubing to, for example, a monitor cell. Save the transport device for use when storing the column. The column is now ready for use.

Equilibrating the column

Tip: *Equilibrate the column a day before usage to save time.*

Ensure that an appropriate pressure limit has been set. Equilibrate the column for first time use, or after long-term storage as follows:

1 One column volume (CV) of low ionic strength buffer at 30 cm/h (1 ml/min for 16/600 or 2.6 ml/min for 26/600).

Recommended running conditions

Flow rate*	30 cm/h (1 ml/min for 16/600 or 2.6 ml/min for 26/600)
Sample volume	0.5% to 4% of the CV (0.6 to 4.8 ml for 16/600 or 1.6 to 12.8 ml for 26/600) ***Note:*** *Sample volume is critical for the separation.*
Sample preparation	Dissolve the sample in running buffer, filter through 0.22 µm filter or centrifuge at 10 000 x g for 10 min
Buffer	0.05 M $NaPO_4$, 0.15 M NaCl, pH 7.2 or select a buffer appropriate for the next purification step. To avoid pH dependent non-ionic interactions with the matrix, include at least 0.15 M salt in the buffer (or use a buffer with equivalent ionic strength).
Regeneration	Regenerate the column after each run with one CV of running buffer at 30 cm/h (1 ml/min for 16/600 or 2.6 ml/min for 26/600)

* Recommended flow rates are valid for H_2O at 25°C.

Read "Optimizing" for information on how to optimize a separation.

2 Two CV of buffer, for example, 0.05 M sodium phosphate, 0.15 M NaCl, pH 7.2 at 50 cm/h (1.6 ml/min for 16/600 or 4.3 ml/min for 26/600).

Note: *When running under cold conditions or using buffer with high viscosity, adjust the flow rate so that the back pressure limit is not exceeded.*

Delivery and storage

The prepacked column is delivered in 0.2 M sodium acetate, 20% ethanol (Superdex 30 and Superdex 75) or 20% ethanol (Superdex 200). If the column needs to be stored for more than two days after use, wash the column with four CV of distilled water, and then equilibrate with four CV of 0.2 M sodium acetate, 20% ethanol or 20% ethanol only, depending on the media. Use the transport device to prevent air from entering the column and destroying the column packing. Connect the transport device to the capillary tubing at the column outlet. Start the pump and fill the device up to approximately 50% of the total device volume.

Daily use

All commonly used aqueous buffers, pH 3 to 12

Cleaning

Acetonitrile, up to 30%
NaOH, up to 1 M
Ethanol, up to 70% (Superdex 30 pg)
Ethanol, up to 24% (Superdex 75 pg and Superdex 200 pg)
Acetic acid, up to 1 M
Isopropanol, up to 30%
Guanidine hydrochloride, up to 6 M
Urea, up to 8 M
Hydrochloric acid, up to 0.1 M (Superdex 30 pg)

Avoid

Unfiltered solutions

Buffers and solvent resistance

De-gas and filter all solutions through 0.22 µm filter to increase the column lifetime. Buffers and solvents with high viscosity will affect the back pressure and flow rate.

Choosing a buffer

Buffer composition does not directly affect the resolution. Select a buffer that is compatible with the stability and activity of the protein to be purified. Buffer concentration must be sufficient to maintain a buffering capacity and a constant pH. Ionic strength should be at least 0.15 M NaCl in the buffer, to avoid non-specific ionic interactions with the matrix.

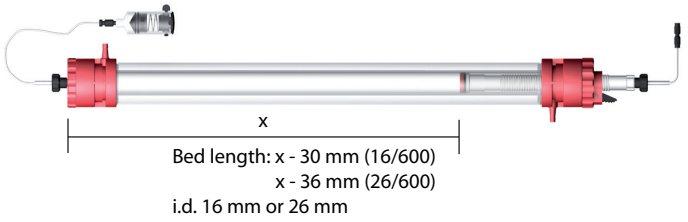


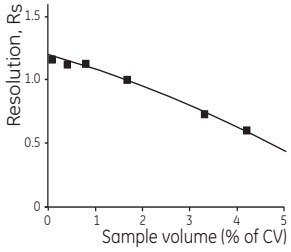
Fig. 3 Dimensions of the column.

Optimizing

Perform a first run as described in "Recommended running conditions". If the results obtained are unsatisfactory, consider the following:

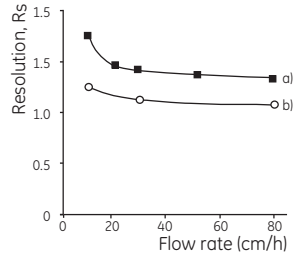
Action	Effect
Decrease flow rate	Improved resolution
Decrease sample volume	Improved resolution

Figures 4 and 5 demonstrate the influence of sample volume and flow rate on the resolution.



Column: HiLoad 16/600 Superdex 200 pg
Sample: Solution of transferrin (M_r 81 000) and IgG (M_r 160 000) by equal weight
Sample concentration: 8 mg/ml
Buffer: 50 mM $NaPO_4$, 0.1 M NaCl, pH 7.2
Flow rate: 30 cm/h (1 ml/min)

Fig. 4 Influence of sample volume on column resolution.



Column: HiLoad 16/600 Superdex 30 pg
Sample: IGF-1 containing monomers and dimers
Sample concentration: a) 1.25 mg/ml
b) 5 mg/ml
Sample volume: 1 ml (0.8% of CV)
Buffer: 50 mM sodium acetate, 0.1 M NaCl, pH 5.0

Fig. 5 Influence of flow rate on the column resolution.

Column resolution is calculated as:

$$R_s = \frac{2(V_{R2} - V_{R1})}{W_{b2} + W_{b1}}$$

where,

V_{R1} = Retention (elution) volume of the first peak

V_{R2} = Retention (elution) volume of the second peak

W_{b1} = Base width of the first peak

W_{b2} = Base width of the second peak

V_R and W_b in same units.

Cleaning-In-Place (CIP)

Regular cleaning

Wash the column with one-half to one CV of 0.5 M NaOH at a flow rate of 25 cm/h (0.8 ml/min for 16/600 or 2.2 ml/min for 26/600) to remove most of the non-specifically bound proteins from the chromatography medium.

After cleaning, immediately equilibrate the column with at least two CV of buffer. Further equilibration is necessary if the buffer contains detergents. Wait until the UV baseline stabilizes before starting a new purification.

More rigorous cleaning

Wash the column at a flow rate of 25 cm/h (0.8 ml/min for 16/600 or 2.2 ml/min for 26/600) at room temperature with the following solutions:

1 Four CV of 1 M NaOH (removes hydrophobic proteins or lipoproteins) followed by four CV of distilled water.

2 One-half CV of 30% isopropanol (removes lipids and very hydrophobic proteins), followed by two CV of distilled water.

Before starting a new purification, equilibrate the column after cleaning with at least five CV of running buffer.

Changing the adapter net ring

After following the cleaning procedures above, if the back pressure of the column remains too high, change the net ring in the column adapter. Follow the instructions below thoroughly since column efficiency is easily impaired if handled without care. Use distilled water as a liquid. For an exploded view of the adapter, see Figure 8.

1 Close the outlet tubing of the column with a stop plug, and mark the level of the chromatography medium surface on the glass tube using a colored pen.

2 Slacken the adapter O-ring slightly by turning the black adjusting knob counter-clockwise.

Note: *It should still seal against the glass wall but allow the adapter to slide. Unscrew the top piece from the column.*

3 Connect the adapter to the pump and start pumping at a flow rate of 30 cm/h (1 ml for 16/600 or 2.6 ml/min for 26/600). Allow the flow to push the adapter upwards.

4 When the glass tube is completely full, take out the adapter and stop the pump. The glass tube should be completely filled with liquid.

5 Change the adapter net ring.

6 To avoid any air bubbles under the net, inject 20% ethanol through the adapter using a syringe.

7 Insert the adapter into the column at an angle of 45°, avoiding air bubbles. Slide the plunger 1 to 2 cm down and tighten the O-ring. Remove excess liquid completely before screwing the top piece onto the column end piece.

8 Remove the syringe and slide down the adapter until it touches the chromatography medium surface. Tighten the O-ring and reconnect the inlet tubing to the system, avoiding air bubbles.

9 Remove the stop plug and start the pump. Increase the flow rate until the medium surface is approximately 3 mm above the pen mark. Stop the pump and close the outlet tubing with the stop plug again.

Note: *This step requires a pump with high flow rate capacity up to a pressure of 0.5 MPa (5 bar).*

10 Disconnect the inlet tubing and slacken the adapter O-ring slightly by turning the adjusting knob counter-clockwise. Press the adapter downwards up to the pen mark. Tighten the O-ring.

Note: *Do not loosen the O-ring too much as this will result in chromatography medium passing through the O-ring.*

11 Reconnect the inlet tubing and avoid introducing air into the system.

Troubleshooting

Symptom	Remedy
Increased back pressure over the column	Clean the column according to the section "Cleaning-In-Place (CIP)"
Loss of resolution and/or decreased sample recovery	Clean the column according to the section "Cleaning-In-Place (CIP)"
Air bubbles in the column	Reverse the direction of flow and pump five CV of degassed water through the column at the same flow rate that was used during the run.
Space between adapter and medium	Close the outlet tubing with the stop plug and then disconnect the inlet tubing. Slacken the O-ring slightly by turning the adjusting knob counter-clockwise and push or screw the adapter down until it touches the medium surface. Tighten the O-ring. To maintain an airtight system, reconnect the inlet tubing immediately.

Testing the column efficiency

GE Healthcare Life Sciences packs columns to the highest standards and each column is thoroughly tested with respect to the number of theoretical plates per meter (N/m) (Fig 6).

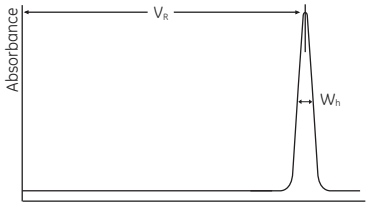


Fig. 6 Column efficiency test

Sample:	2% acetone in water
Sample volume:	200 µl (16/600) and 500 µl (26/600)
Eluent:	Distilled water
Flow rate:	60 cm/h 2.0 ml/min (16/600) 5.3 ml/min (26/600)
Temperature:	Room temperature (25°C)

Column efficiency is calculated using the equation:

$$N/m = 5.54 \times \left(\frac{V_R}{W_h}\right)^2 / L$$

where,

V_R = Peak retention (elution) volume
W_h = Peak width at half peak height
L = Bed height (meter)
V_R and W_h have the same units.

Adjusting pressure limits in chromatography system software

Pressure generated by the flow, through a column, affects the packed bed, and the column hardware, see Figure 7. Increased pressures might be generated when running/using one or a combination of the following conditions:

- High flow rates
- Buffers or sample with high viscosity
- Low temperature
- A flow restrictor

Note: *Exceeding the pressure limits (see Table 2) will damage the column.*

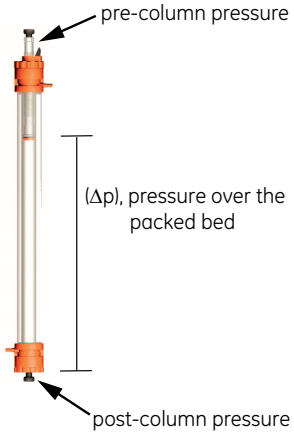


Fig. 7 Pre-column and post-column measurements.

ÄKTA™ avant

The system will automatically handle all pressure limits, which facilitates an optimal functionality without any need of adjustments.

ÄKTAexplorer™, ÄKTApurifier™, ÄKTAFPLC™ and other systems with pressure sensor in the pump

To obtain optimal functionality, the pressure limits in the software may be adjusted according to the following procedure:

1 Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note the pressure as *total system pressure*.

2 Disconnect the tubing and run the pump at the same flow rate used in step 1. Note that there will be a drip from the column valve. Note the pressure during this operation as *measured pressure*.

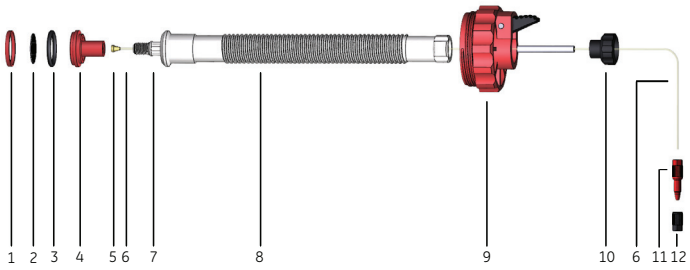
3 Calculate *column pressure limit* as a sum of *total system pressure* and Δp (*pressure over the packed bed*) (see Table 2).

4 Replace the *column pressure limit* in the software with the calculated value.

Calculate *post-column pressure* as the difference between *total system pressure* and *measured pressure*.

Column hardware pressure limit (see Table 2) must never exceed the sum of *post-column pressure* and Δp.

Note: *Repeat the procedure each time the parameters are changed.*



1 Net ring
2 Support screen
3 O-ring
4 Plunger
5 Ferrule
6 Capillary tubing
7 Inner shaft
8 Adapter shaft
9 Top end cap
10 Adjusting knob
11 HiTrap/HiPrep, 1/16" male connector for ÄKTA design
12 Stop plug

Fig. 8 Exploded view of the XK column adapter used at the top of the HiLoad column.

Intended use

HiLoad Superdex columns are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Ordering information

Product	Pack size	Code No.
HiLoad 16/600 Superdex 30 prep grade	1 x 120 ml	28-9893-31
HiLoad 26/600 Superdex 30 prep grade	1 x 320 ml	28-9893-32
HiLoad 16/600 Superdex 75 prep grade	1 x 120 ml	28-9893-33
HiLoad 26/600 Superdex 75 prep grade	1 x 320 ml	28-9893-34
HiLoad 16/600 Superdex 200 prep grade	1 x 120 ml	28-9893-35
HiLoad 26/600 Superdex 200 prep grade	1 x 320 ml	28-9893-36

Accessories	No. supplied	Code No.
Accessory kit XK 16*	1	28-9899-78
Accessory kit XK 26*	1	28-9899-79
Support screen XK 16	5	19-0651-01
Support screen XK 26	5	18-9377-01
Net ring (10 µm) XK 16	5	18-8761-01
Net ring (10 µm) XK 26	5	18-8760-01
O-ring XK 16	5	19-0163-01
O-ring XK 26	5	28-9782-27
Stop plug female, 1/16"	5	11-0004-64
HiTrap/HiPrep 1/16" male connector for ÄKTA design	8	28-4010-81
Transport device	1	18-1176-43

* Accessory kits XK 16 and XK 26 are suitable for repacking purposes and contain: 2 support screens, 5 net rings, 2 O-rings, 2 stop plugs, 10 HiTrap/HiPrep 1/16" male connectors for ÄKTA design, and 1 tool for dismantling.

Related literature	Code No.
Gel Filtration Handbook, Principles and Methods	18-1022-18
Gel Filtration Columns and Media, Selection Guide	18-1124-19
Prepacked chromatography columns for ÄKTA systems, Selection Guide	28-9317-78

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