HiLoad 16/600 Superose 6 prep grade

Instructions for Use

Introduction

HiLoad™ 16/600 Superose™ 6 prep grade is a prepacked XK column designed for preparative size exclusion chromatography.

Superose 6 prep grade is a resin of cross-linked agarose optimised for high performance size exclusion chromatography of biomolecules.

The size and distribution of the particles allow high flow, high efficiency, good capacity, and negligible ionic interactions at an eluent ionic strength of above 0.05 M. Some hydrophobic interactions have been recognized, for example, some compounds can be eluted later than predicted. These interactions can be of considerable value to the resolution.

Table 1. Contents of the delivery box

Component	No. supplied	Sequence in Fig. 1
Transport device	1	1
1/16" male connectors	2	2
Stop plug	1	2
Instructions	1	3
HiLoad column	1	4

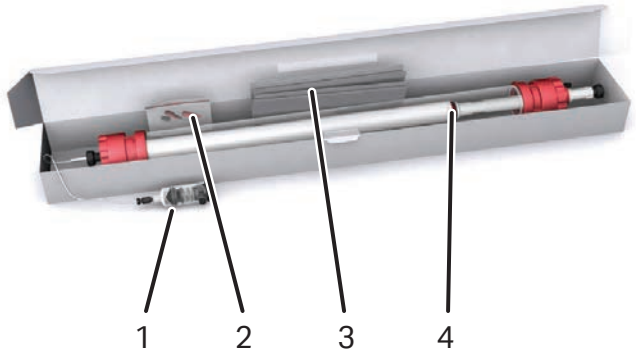


Fig 1. Package includes HiLoad column, transport device, two connectors, one stop plug, and instructions

Table 2. HiLoad column characteristics

Matrix	Cross-linked agarose, 6%, spherical
Particle size, d50v[1]	~30±10 µm
Fractionation range (Mr)	~5000 to 5×106
Exclusion limit globular proteins	~4×107
Column volume[2]	120 to 124 mL
Sample volume[3]	Up to 5 mL
Recommended flow rate	1 mL/min at room temperature
Maximum flow rate	1.6 mL/min at room temperature
Theoretical plates	> 10 000 m-1
Maximum pressure over the packed bed during operation, Δp	0.3 MPa, 3 bar, 42 psi
Column hardware pressure limit[4]	0.5 MPa, 5 bar, 73 psi
pH stability: operational[5] CIP[6]	3 to 12 1 to 14
Storage	20% ethanol at room temperature

[1] Median particle size of the cumulative volume distribution.

[2] The surface of the resin is not directly visible at the bottom. Therefore, when calculating the total column volume, calculate the height of the resin from the lowest part of the bottom piece to the surface of the resin/ adapter. Deduct 30 mm.

[3] 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 can be necessary to decrease both).

[4] See *Adjusting pressure limits in chromatography system software, on page 4*.

[5] pH range where resin can be operated without significant change in function.

[6] pH range where resin can be subjected to cleaning or sanitization-in-place without significant change in function.

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 us 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.*

Make sure 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 1 mL/min.
2. Two CV of buffer, for example, 0.05 M phosphate buffer, 0.15M NaCl, pH 7.2 at 1.6 mL/min.

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.*

Recommended running conditions

Flow rate[1]	1 mL/min
Sample volume	0.5% to 4% of the CV; 0.6 to 4.8 ml 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 phosphate buffer, 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 1 mL/min.

[1] Recommended flow rates at room temperature for solutions with viscosity similar to water.

Read *Optimizing, on page 2* for information on how to optimize a separation.

Delivery and storage

The prepacked column is delivered in 20% ethanol. 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 20% ethanol. 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

Commonly used aqueous buffers, pH 3 to 12

Cleaning

Acetonitrile, up to 30%

NaOH, up to 1 M

Ethanol, up to 24%

Acetic acid, up to 1 M

Isopropanol, up to 24%

Avoid

Unfiltered solutions

Buffers and solvent resistance

De-gas and filter all solutions through a 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 media.

Optimizing

Perform a first run as described in *Recommended running conditions, on page 2*. If the results obtained are unsatisfactory, consider the following:

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

Figures 2 and 3 below demonstrate the influence of sample volume and flow rate on the resolution.

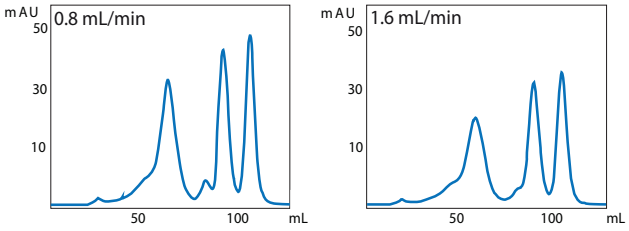


Fig 2. Comparison of protein separation on HiLoad 16/600 Superose 6 prep grade at different flow rates.

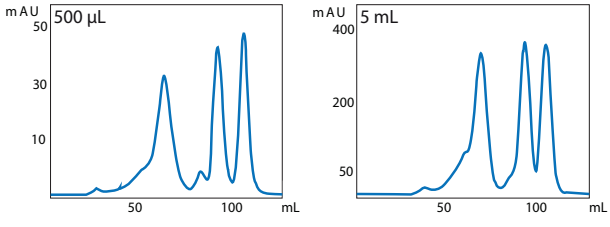


Fig 3. Comparison of protein separation on HiLoad 16/600 Superose 6 prep grade using different sample volumes.

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

VR and Wb 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 0.8 mL/min to remove most of the non-specifically bound proteins from the chromatography resins. 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 0.8 mL/min 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 carefully since column efficiency is easily impaired if handled without care. Use distilled water as a liquid. For an exploded view of the adapter, see *Fig. 7, on page 5*.

Step	Action
1	Close the outlet tubing of the column with a stop plug, and mark the level of the chromatography resin 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 1 mL/min. 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 resin surface. Tighten the O-ring and reconnect the inlet tub-ing to the system, avoiding air bubbles.
9	Remove the stop plug and start the pump. Increase the flow rate until the resin 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 down-wards up to the pen mark. Tighten the O-ring. ***Note:*** *Do not loosen the O-ring too much as this will result in chromatography resin 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), on page 3*.
Loss of resolution and/or decreased sample recovery	Clean the column according to the section *Cleaning-In-Place (CIP), on page 3*.
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 resin	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 resin surface. Tighten the O-ring. To maintain an airtight system, reconnect the inlet tubing immediately.

Testing the column efficiency

Cytiva 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 below).

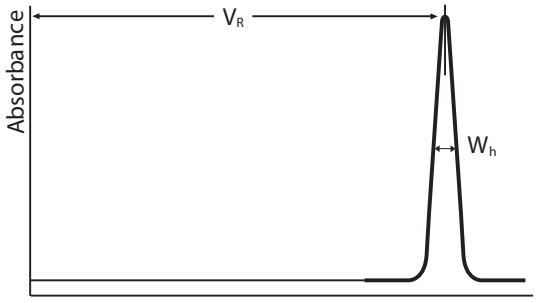


Fig 4. Column efficiency test

Sample:	2% acetone in water
volume: Eluent:	200 µl
Flow rate:	Distilled water 2 0 mL/min
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 *Fig. 5, on page 4*. 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
- Long narrow tubing

Note: *Exceeding the pressure limits (see Table 2, on page 1) will damage the column.*

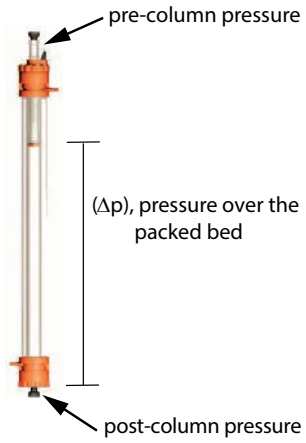


Fig 5. Pre-column and post-column measurements.

ÄKTA avant and ÄKTA pure

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

ÄKTA start 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:

Step	Action
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, on page 1*).
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, on page 1*) must never exceed the sum of *post-column pressure* and Δp. ***Note:*** *Repeat the procedure each time the parameters are changed*

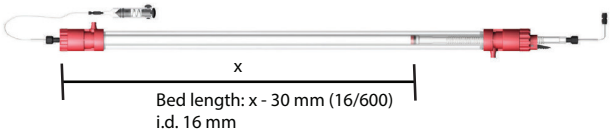
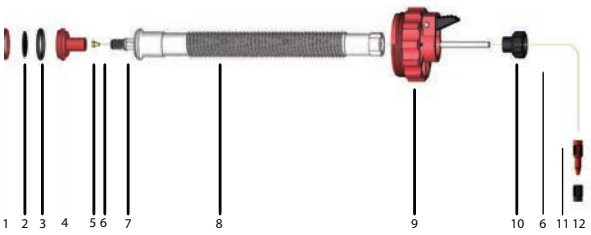


Fig 6. Dimensions of the column



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

Intended use

HiLoad Superose 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	Packet size	Code no.
HiLoad 16/600 Superose 6 prep grade	1 x 120 mL	29323952
HiLoad 16/600 Superdex™ 30 prep grade	1 x 120 mL	28989331
HiLoad 26/600 Superdex 30 prep grade	1 x 320 mL	28989332
HiLoad 16/600 Superdex 75 prep grade	1 x 120 mL	28989333
HiLoad 26/600 Superdex 75 prep grade	1 x 320 mL	28989334
HiLoad 16/600 Superdex 200 prep grade	1 x 120 mL	28989335
HiLoad 26/600 Superdex 200 prep grade	1 x 320 mL	28989336

Related Products	Packet size	Code no.

Accessories	No. supplied	Code No.
Accessory kit XK 16[1]	1	28989978
Support screen XK 16	5	19065101
Net ring (10 µm) XK 16	5	18876101
O-ring XK 16	5	19016301
Stop plug female, 1/16"	5	11000464
HiTrap/HiPrep 1/16" male connector for ÄKTA design	8	28401081
Transport device	1	18117643

[1] Accessory kit XK 16 is 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.
Size Exclusion Chromatography Handbook, Principles and Methods	18102218
Size Exclusion Chromatography Columns and Resins, Selection Guide	18112419
Prepacked chromatography columns for ÄKTA systems, Selection Guide	28931778

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