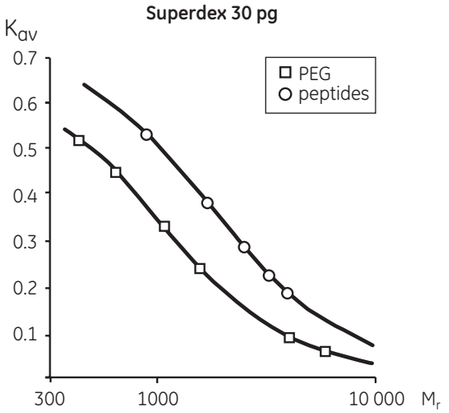
GE Healthcare

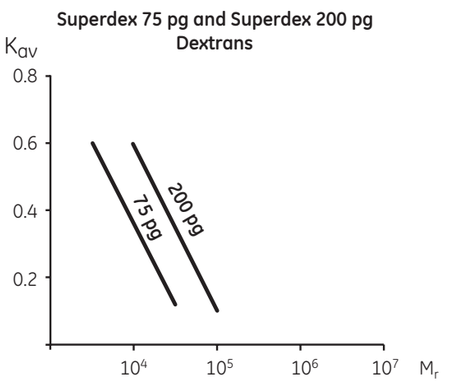
Instructions 71-5020-20 AF **High Performance Columns**

HiLoad 16/60 and 26/60 Superdex 30 prep grade
HiLoad 16/60 and 26/60 Superdex 75 prep grade
HiLoad 16/60 and 26/60 Superdex 200 prep grade

Introduction

HiLoad™ 16/60 and 26/60 Superdex™ 30 prep grade, Superdex 75 prep grade, and Superdex 200 prep grade are prepacked XK columns designed for preparative gel filtration chromatography separations. Superdex prep grade (pg) is produced by covalent bonding of dextran to highly cross-linked agarose. The separation properties of these media are determined by the dextran component. Steep selectivity curves give excellent resolving power for peptides and proteins in the molecular weight ranges, M_r < 10 000 (Superdex 30 pg), M_r 3 000–70 000 (Superdex 75 pg), and M_r 10 000–600 000 (Superdex 200 pg), see Figure 1. The media combines high mechanical strength with high hydrophilicity, allowing high flow rates, and minimal non-specific interactions.





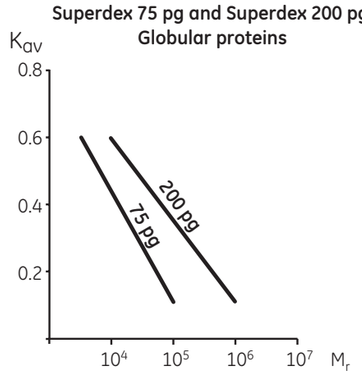


Fig 1. Selectivity curves from Superdex 30 pg, Superdex 75 pg and Superdex 200 pg.

Column data

Matrix	Dextran covalently bound to highly cross-linked agarose
Mean particle size	34 µm
Separation range (M_r)	<10 000 (Superdex 30 pg)
globular proteins	3×10^3–7×10^5 (Superdex 75 pg) 1×10^4–6×10^5 (Superdex 200 pg)
dextrans	5×10^2–3×10^4 (Superdex 75 pg) 1×10^3–1×10^5 (Superdex 200 pg)
Column volume[1]	120–124 ml (XK 16/60) 319–330 ml (XK 26/60)
Sample volume[2]	Up to 5 ml (XK 16/60) Up to 13 ml (XK 26/60)
Recommended flow rate	10–50 cm/h at room temperature (0.3–1.6 ml/min for XK 16/60 or 0.9–4.4 ml/min for XK 26/60)
Theoretical plates	>13 000 m^{-1}
Maximum pressure over the packed bed during operation[3]	0.3 MPa, 3 bar, 42 psi
HiLoad column hardware pressure limit[3]	0.5 MPa, 5 bar, 73 psi
pH stability	
long term and working range	3–12
short term	1–14
Storage	20% ethanol

1 The medium surface is not directly visible at the bottom piece. Therefore, when calculating the total column volume, measure the height from the lowest part of the bottom piece to the medium/adaptor surface. For HiLoad 16/10 deduct 30 mm, and for HiLoad 26/10 deduct 36 mm.

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

3 In exceptional circumstances, the packed bed can withstand pressures of up to 0.4 MPa, 4 bar, 56 psi for short periods. Many chromatography systems are equipped with pressure gauges to measure pressure at a particular point in the system, usually just after the pumps. The pressure measured here is the sum of pre-column pressure, pressure drop over the medium bed, and post-column pressure. This is always higher than the pressure drop over the bed alone. Keeping the pressure drop over the bed below 2 bar is recommended. Setting the upper limit of the pressure gauge to 2 bar will ensure that the pump shuts down before the medium is over-pressured. If necessary, post-column pressure of up to 3 bar can be added to the limit without exceeding the column hardware limit. To determine post-column pressure, proceed as follows:

To avoid breaking the column, post-column pressure must never exceed 2 bar.

1. Connect a piece of tubing in place of the column.
2. Run the pump at the maximum flow you intend to use for chromatography. Use a buffer with the same viscosity as you intend to use for chromatography. Note the back pressure as total pressure.
3. Disconnect the tubing and run at the same flow rate used in step 2. Note this back pressure as pre-column pressure.
4. Calculate post-column pressure as total pressure minus pre-column pressure.

If post-column pressure is higher than 2 bar, take steps to reduce it (shorten tubing, clear clogged tubing, or change flow restrictors), and perform steps 1–4 again until the post-column pressure is below 2 bar. Note post-column pressure when it has reached a satisfactory level, add 3 bar to this value, and set this as the upper pressure limit on the chromatography system.

First-time use

Connecting the column

1. Before connecting the column to a chromatography system, start the pump to remove all air from the system, particularly in tubing and valves.
2. Stop the pump.
3. Mount the column vertically, remove the domed nut, and connect the inlet tubing to the system "drop-to-drop".
4. Remove the transport syringe and connect the column outlet tubing to, for example, a monitor cell. Save the transport syringe for use when storing the column. The column is now ready for use.

Equilibration of the column

Ensure 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 of low ionic strength buffer at 30 cm/h (1 ml/min for XK 16/60 or 2.6 ml/min for XK 26/60).
2. Two column volumes of buffer, e.g. 0.05 M sodium phosphate, 0.15 M NaCl, pH 7.2 at 50 cm/h (1.6 ml/min for XK 16/60 or 4.3 ml/min for XK 26/60).

Try these conditions first

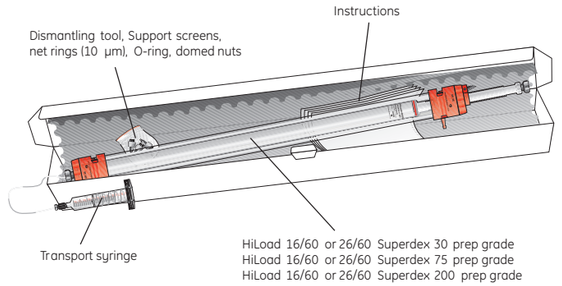
Flow rate: 30 cm/h (1 ml/min for XK 16/60 or 2.6 ml/min for XK 26/60).

Sample volume: 1% of the column volume (1.2 ml for XK 16/60 or 3.2 ml for XK 26/60).

Buffer: 0.05 M sodium phosphate, 0.15 M NaCl, pH 7.2 or select the buffer in which the sample should be in for the next step.

To avoid pH dependent non-ionic interactions with the matrix, include at least 0.15 M salt in the buffer (or use buffer with equivalent ionic strength).

Read the section "Optimization" for information on how to optimize a separation.



Equilibration before a new run

Regenerate the column after each run with one column volume of buffer at 30 cm/h (1 ml/min for XK 16/60 or 2.6 ml/min forXK 26/60).

Buffers and solvent resistance

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

Daily use

All commonly used aqueous buffers, pH 3–12

Cleaning

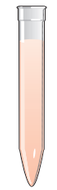
Acetonitrile, up to 30%
Sodium hydroxide, up to 1 M
Ethanol, up to 70% (Superdex 30 pg)
Ethanol, up to 24% (Superdex 75 pg and Superdex 200 pg)
Acetic acid, up to 1 M
Isopropanol, up to 30%
Guanidine hydrochloride, up to 6 M
Urea, up to 8 M
HCl, up to 0.1 M (Superdex 30 pg)

Avoid

Unfiltered solutions

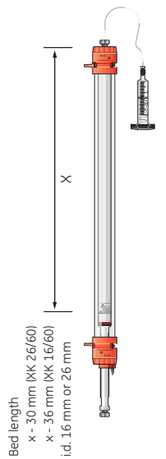
Sample recommendations

Recommended sample load	0.5–4% of the column volume (0.6–4.8 ml for XK 16/60, or 1.6–12.8 ml for XK 26/60) **Note:** The sample volume is critical for the separation.
Preparation	Dissolve the sample in start buffer, filter through 0.22 µm filter, or centrifuge at 10 000 × g for 10 min.



Delivery and storage

The prepacked column is supplied in 20% ethanol. If the column is to be stored more than 2 days after use, wash the column with four column volumes of distilled water, and then equilibrate with four column volumes of 20% ethanol. To avoid air bubble formation in the column, use the transport syringe. Connect the transport syringe to the capillary tubing at the column outlet. Start the pump, and fill the syringe to approximately 50% of the total syringe volume.



Choice of buffer

Selection of buffering ion does not directly affect resolution. Select a buffer in which the purified product should be collected, and which is compatible with protein stability and activity. Buffer concentration must be sufficient to maintain buffering capacity and constant pH.

Ionic strength should be at least 0.15 M NaCl in the start buffer, to avoid non-specific ionic interactions with the matrix.

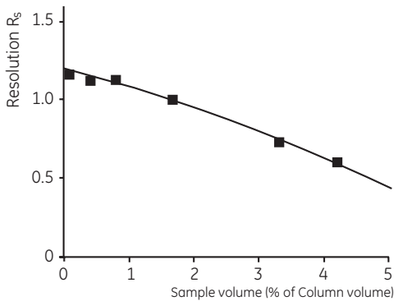
Optimization

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

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

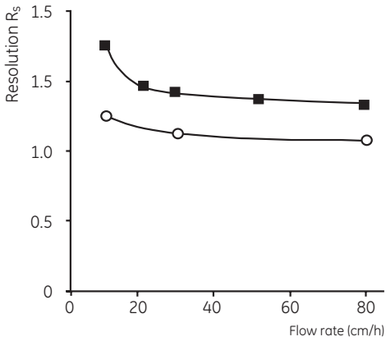
HiLoad™

Fig. 2 and 3 show the influence of sample volume and flow rate on the resolution.



Column:	HiLoad 16/60 Superdex 200 pg
Buffer:	50 mM $NaPO_4$, 0.1 M NaCl, pH 7.2
Sample:	Solution of transferrin (M_r 81 000) and IgG (M_r 160 000) by equal weight
Sample concentration:	8 mg/ml
Linear flow rate:	30 cm/h (1 ml/ml)

Fig 2. Influence of sample volume on the resolution.



Column:	HiLoad 16/60 Superdex 30 pg
Buffer:	50 mM sodium acetate, 0.1 M NaCl, pH 5.0
Sample:	IGF-1 containing monomers and dimers
Sample volume:	1 ml (0.8% of column volume)
Sample concentration:	a) 1.25 mg/ml b) 5 mg/ml

Fig 2. Influence of flow rate on the resolution.

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

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 column volume of 0.5 M NaOH at a flow rate of 25 cm/h (0.8 ml/min for XK 16/60 or 2.2 ml/min for XK 26/60) to remove most non-specifically adsorbed proteins to the medium.

After cleaning, immediately equilibrate the column with at least two column volumes of buffer. Further equilibration is necessary if your buffer contains detergent. Wait until the UV base line stabilizes before applying next sample.

More rigorous cleaning

Wash the column at a flow rate of 25 cm/h (0.8 ml/min forXK 16/60 or 2.2 ml/min for XK 26/60) at room temperature with the following solutions:

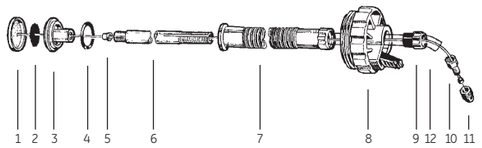
1. Four column volumes of 1 M NaOH (removal of hydrophobic proteins or lipoproteins) followed by four column volumes of distilled water.
2. One-half column volume of 30% isopropanol (removal of lipids and very hydrophobic proteins), followed by two column volumes of distilled water.

If a new purification is to be run, equilibrate the column after cleaning with at least five column volumes of buffer.

Changing the adaptor net ring

If, following cleaning procedures above, back pressure remains too high, change the net ring in the column adaptor. Follow the instructions below thoroughly since column efficiency is easily impaired if careless measures are taken. Use distilled water as eluent.

1. Close the outlet tubing of the column with a domed nut, and mark the level of the medium surface on the glass tube using a coloured pen.
2. Slacken the adaptor O-ring slightly by turning the black adjusting knob counter-clockwise. **Note:** It should still seal against the glass wall but allow the adaptor to slide. Unscrew the top piece from the column.
3. Connect the adaptor to the pump and start pumping at a flow rate of 30 cm/h (1 ml for XK 16/60 or 3 ml/min forXK 26/60). Let the flow push the adaptor upwards.
4. When the glass tube is completely full, take out the adaptor and stop the pump. The glass tube should remain completely full of liquid while changing the adaptor net ring.
5. To avoid getting air bubbles under the net, injection of 20% ethanol through the adaptor by a syringe is recommended.
6. Insert the adaptor into the column at an angle of 45°, avoiding air bubbles. Slide the plunger 1–2 cm down and tighten the O-ring. Remove excess liquid completely before screwing the top piece onto the column end piece.
7. Remove the syringe and slide down the adaptor until it touches the medium surface. Tighten the O-ring and re-connect the inlet tubing to the system, avoiding air bubbles.
8. Remove the domed nut 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 domed nut again. **Note:** Step 8 requires a pump with high flow rate capacity up to a pressure of 0.5 MPa, 5 bar.
9. Disconnect the inlet tubing and slacken the adaptor O-ring slightly by turning the adjusting knob counter-clockwise. Press the adaptor downwards to the pen mark. Tighten the O-ring. **Note:** Do not to loosen the O-ring too much as this will result in gel passing the O-ring.
10. Re-connect the inlet tubing, and avoid introducing air into the system.



1. Net ring
2. Support screen
3. Plunger
4. O-ring
5. Sealing plug
6. Inner adjusting
7. Outer adjusting tube
8. Top piece
9. Adjusting knob
10. Capillary tubing
11. Domed nut
12. Protection tubing

Troubleshooting

Symptom	Remedy
Increased backpressure 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 in the column	Reverse flow direction and pump five column volumes of well de-gassed water through the column at a flow rate of 150 cm/h (5 ml/min for XK 16/60 or 13 ml/min for XK 26/60).
Space between adaptor and medium	Close the outlet tubing with the domed nut and then disconnect the inlet tubing. Slacken the O-ring slightly by turning the adjusting knob counter-clockwise and push or screw the adaptor down until it touches the medium surface. Tighten the O-ring. To maintain an airtight system, reconnect the inlet tubing immediately.

Column efficiency test

GE Healthcare packs columns to the highest standards, and each column is thoroughly tested with regard to the number of theoretical plates per metre (H-1), see Figure 4.

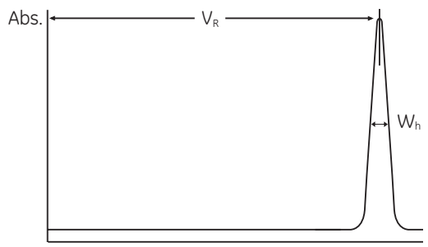


Fig 4. Column efficiency test.

Sample	Acetone 20 mg/ml
Sample volume	200 µl (XK 16/60) and 500 µl (XK 26/60)
Eluent	Distilled water
Linear flow rate	60 cm/h

Column efficiency is calculated using the equation:

$H^{-1} = 5.54 (V_R/w_h)^2 \ 1000/L$

where,

V_R = Peak retention (elution) volume
w_h = Peak width at half peak heigh
L = Bed height (mm)
V_R and w_h in same units

Ordering information

Product	No. per pack	Code No.
HiLoad 16/60 Superdex 30 prep grade	1 (120 ml)	17-1139-01
HiLoad 26/60 Superdex 30 prep grade	1 (320 ml)	17-1140-01
HiLoad 16/60 Superdex 75 prep grade	1 (120 ml)	17-1068-01
HiLoad 26/60 Superdex 75 prep grade	1 (320 ml)	17-1070-01
HiLoad 16/60 Superdex 200 prep grade	1 (120 ml)	17-1069-01
HiLoad 26/60 Superdex 200 prep grade	1 (320 ml)	17-1071-01

Accessories

Product	No. supplied	Code No.
Dismantling tool*	1	–
Support screen XK 16*	2	19-0651-01
Support screen XK 26*	2	18-9377-01
Net ring (10 µm) XK 16*	2	18-8761-01
Net-ring (10 µm) XK 26*	2	18-8760-01
Transport syringe*	1	18-1017-61
O-ring XK 16*	1	19-0163-01
O-ring XK 26*	1	19-0688-01
Domed nut	2	18-2450-01
Union M6 female/1/16" male (for connection to ÄKTAdesign™ systems)	2	18-3858-01

* Included in HiLoad 16/60 and/or 26/60 Superdex prep grade

Related printed literature

Designation	No. per pack	Code No.
Gel Filtration Handbook, Principles and Methods	1	18-1022-18
Gel Filtration Columns and Media, Selection Guide	1	18-1124-19

Further information

Check *www.chromatography.amershambiosciences.com* for more information. For example are reference lists available.

Reference list*	Code No.
HiLoad Superdex 30 prep grade	18-1156-94
HiLoad Superdex 75 prep grade	18-1156-95
HiLoad Superdex 200 prep grade	18-1156-96

Note: Only available on the web site, not in printed versions.

www.chromatography.amershambiosciences.com
www.gehealthcare.com

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

imagination at work

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare UK Ltd
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue, P.O. Box 132, Piscataway, NJ 08855-1327, USA

GE Healthcare Bio-Sciences KK
Sanken Bldg, 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan

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