

## HiLoad™ Superdex™ 30 prep grade HiLoad Superdex 75 prep grade HiLoad Superdex 200 prep grade

## Data File

### Pre-packed gel filtration columns

HiLoad™ columns are XK laboratory columns pre-packed with separation media for gel filtration, ion exchange, or hydrophobic interaction chromatography. Six of the current range of HiLoad columns are packed with Superdex™ prep grade gel filtration media (Fig. 1).\*

Superdex prep grade is a modern preparative gel with a composite matrix of dextran and highly cross-linked agarose. The steep selectivity of the dextran component and the high chemical and physical stability of the agarose give high resolution separations at flow rates up to 50 cm/h.

HiLoad columns offer a number of significant advantages for high resolution preparative work:

- Pre-packed for convenience and reproducibility
- Columns run on a variety of chromatography systems
- High resolution separation of biomolecules
- High capacity at high flow rates
- High chemical stability
- Separations can be scaled up to production levels

Three types of Superdex prep grade are available in HiLoad columns; Superdex 30 prep grade, Superdex 75 prep grade and Superdex 200 prep grade. All are pre-packed to 60 cm bed heights in 16 mm or 26 mm diameter columns.

Each column is expertly packed and individually tested. This combination of pre-packed convenience and reproducibility make HiLoad Superdex prep grade columns a confident choice for fast, high resolution gel filtration at preparative scale, especially when the purification process is to be scaled up.

The columns run with a wide variety of equipment, including simple pump-based configurations, GradiFrac™, FPLC®, HPLC, and BioPilot® systems.

\* HiLoad columns for ion exchange and HIC are described in a separate Data File with Article No. 18-1100-53.



Fig. 1. HiLoad™ Superdex™ 30, 75 and 200 prep grade columns bring convenience and high resolution to preparative gel filtration. Each is available in two column sizes, HiLoad 16/60 and HiLoad 26/60.

### HiLoad Superdex 30 prep grade, Superdex 75 prep grade and Superdex 200 prep grade

Superdex gel filtration media are produced by the covalent binding of dextran to highly cross-linked porous agarose beads. The selectivity of Superdex 30 prep grade (Fig. 2) is between that of Sephadex® G-25 and G-50, while Superdex 75 prep grade and Superdex 200 prep grade have selectivities similar to those of Sephadex G-75 and Sephadex G-200 respectively (Fig. 3) (1).

Steep selectivity curves give unmatched resolution for biomolecules in the molecular weight range up to 10 000 for Superdex 30 prep grade, 3 000–70 000 for Superdex 75 prep grade, and 10 000–600 000 for Superdex 200 prep grade (Figs. 4 and 5).

Moreover, the mean particle size of 34 µm and narrow particle size distribution of Superdex prep grade media give good separation performance without creating high back pressure.

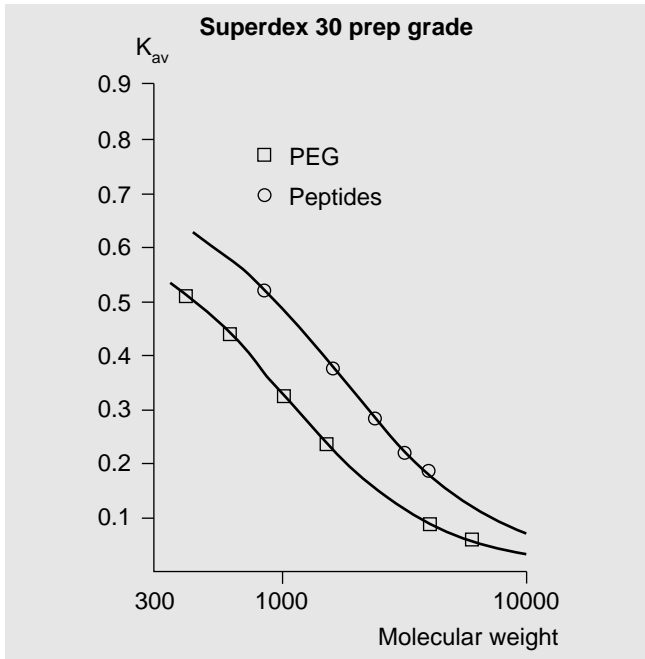


Fig. 2. Selectivity curves for Superdex 30 prep grade.

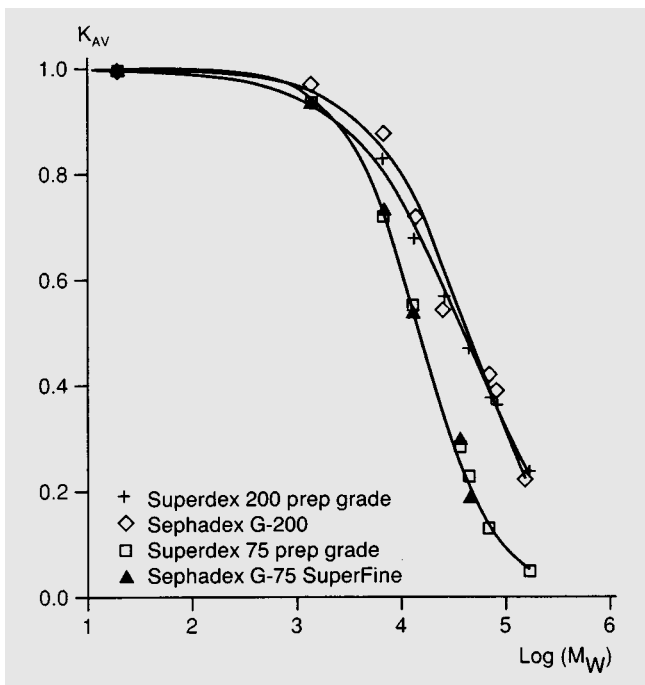


Fig. 3. Selectivity curves for Sephadex® and Superdex prep grade media.

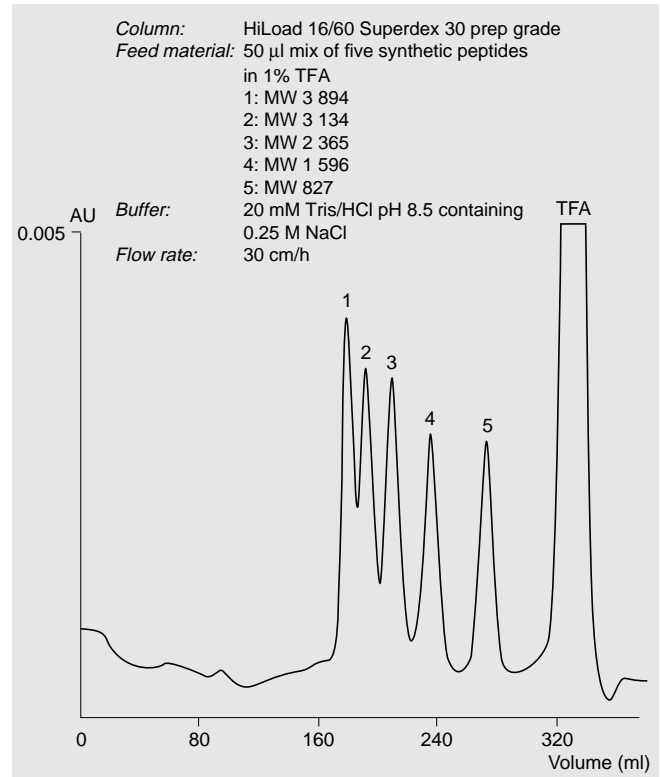


Fig. 4. Separation of test substances on HiLoad 16/60 Superdex 30 prep grade. Superdex 30 prep grade is optimized for proteins/peptides below 10 000.

Table 1 summarizes the characteristics of the media and columns.

Table 1. Characteristics of HiLoad Superdex 30 prep grade, Superdex 75 prep grade and Superdex 200 prep grade.

Separation range (MW)	
peptides/ proteins	
Superdex 30 prep grade:	up to 10 000
Superdex 75 prep grade:	3 000–70 000
Superdex 200 prep grade:	10 000–600 000
Mean particle size:	34 µm
Bead size range:	24–44 µm >75%
Bead structure:	composite of cross-linked agarose and dextran, spherical
Column inner diameter:	16 mm or 26 mm
Bed height:	60 cm
Bed volumes:	120–124 ml (16 mm i.d.), 319–330 ml (26 mm i.d.)
Theoretical plates per meter (N/m)	>13 000
Max. back pressure:	3 bar
Recommended flow rate:*	10–50 cm/h
Solutions in which the gel is stable:	all commonly used buffers 1 M acetic acid 8 M urea 6 M guanidine hydrochloride 30% isopropanol 30% acetonitrile 70% ethanol 1 M sodium hydroxide 0.1 M hydrochloric acid
pH stability working range:	3–12
cleaning-in-place:	1–14

\* At room temperature in aqueous buffer.

Column: a) HiLoad 16/60 Superdex 75 prep grade  
 b) HiLoad 16/60 Superdex 200 prep grade

Sample: 1. Myoglobin 1.5 mg/ml Mw 17 000  
 2. Ovalbumin 4 mg/ml Mw 43 000  
 3. Albumin 5 mg/ml Mw 67 000  
 4. IgG 0.2 mg/ml Mw 158 000  
 5. Ferritin 0.24 mg/ml Mw 440 000

Sample vol: 0.5 ml  
 Buffer: 0.15 M NaCl, 0.05 M phosphate buffer pH 7.0  
 0.01% sodium azide

Flow rate: 1.5 ml/min

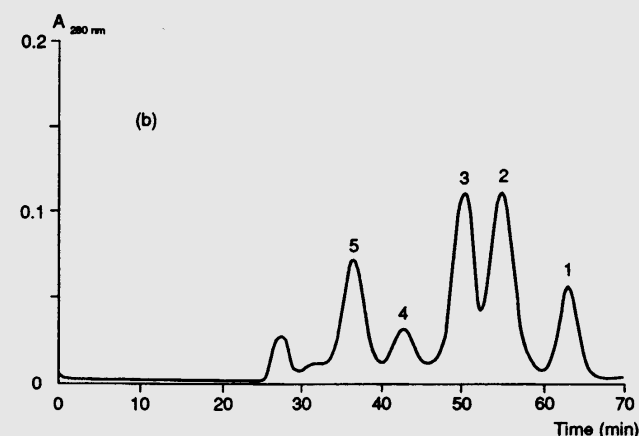
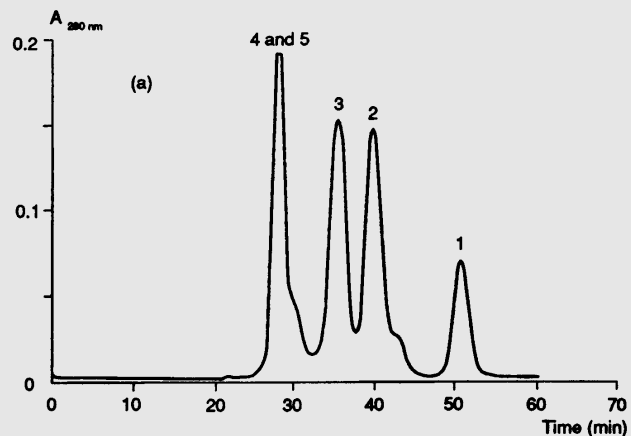


Fig. 5. Comparison of the selectivity of Superdex 75 prep grade and Superdex 200 prep grade for model proteins. Superdex 75 prep grade (a) gives excellent resolution of the three proteins in the mol. wt. range 17 000 to 67 000 while the two largest elute together in the void volume. Superdex 200 prep grade (b) resolves these two largest proteins completely. The three smaller proteins are not resolved quite as well as the larger ones or as in (a). The void volume peak at 28 minutes in (b) is caused by protein aggregates.

## Media characteristics

### Chemical stability

Separation media based on highly cross-linked agarose are very stable and can be run at high flow rates. Figure 6 shows how resolution ( $R_s$ ) is affected by changing flow rate and sample volume. Low sample volumes give high resolution even at high flow rates.

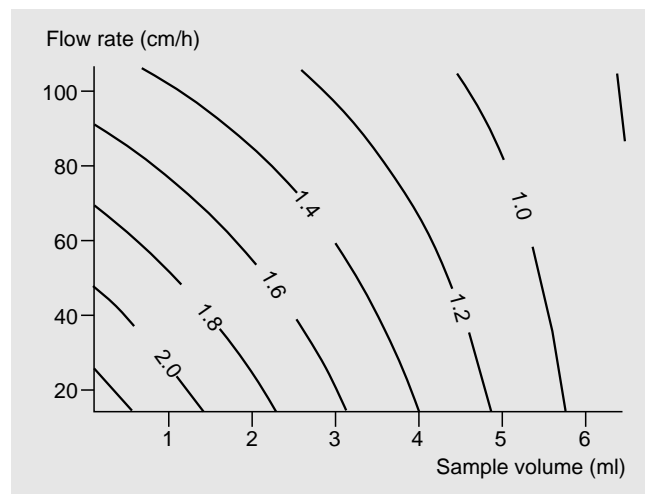


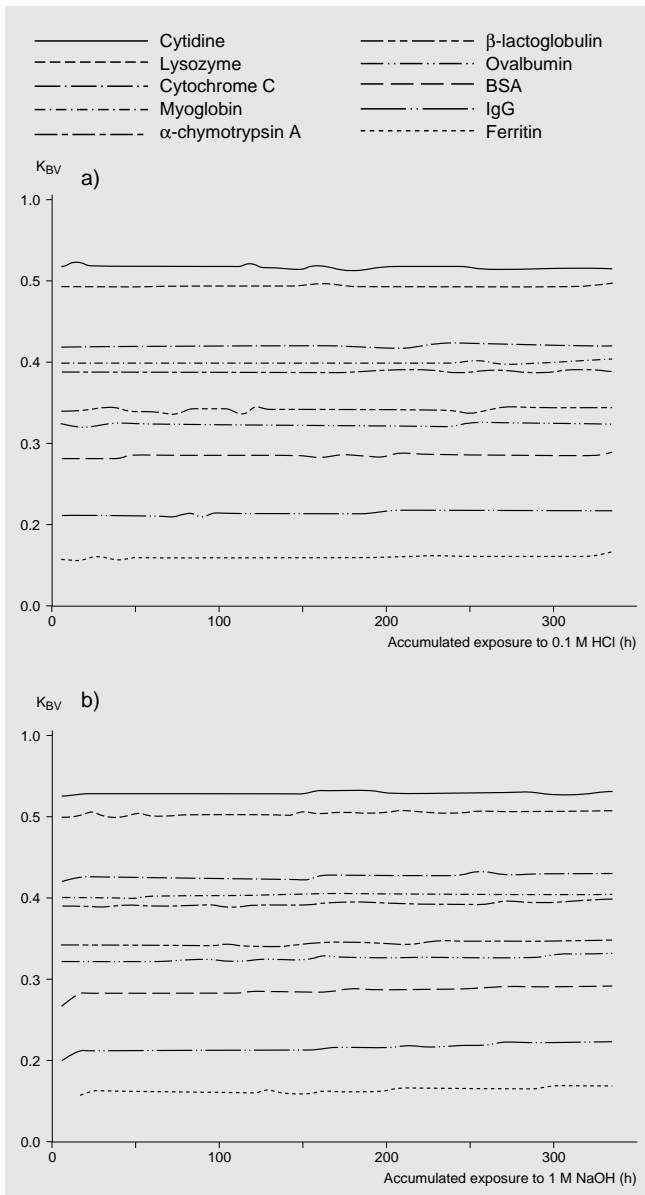
Fig. 6. Effect on resolution ( $R_s$ ) between ribonuclease A and  $\beta$ -lactoglobulin as a function of sample volume and flow rate for HiLoad 16/60 Superdex 30 prep grade.

Extensive studies, including long term exposure to harsh chemical treatment, have demonstrated that Superdex prep grade has extremely high chemical stability (2, 3). Figure 7 illustrates the long term stability of Superdex 200 prep grade to 0.1 M HCl and 1 M NaOH, a feature that is important for cleaning-in-place (CIP) procedures.

Superdex 30 prep grade, Superdex 75 prep grade and Superdex 200 prep grade may be used in aqueous solutions over the range pH 3–12 for continuous operation and over the range pH 1–14 for CIP. Chaotropic agents such as 6 M guanidine hydrochloride or 8 M urea, detergents (ionic and non-ionic) and polar organic solvents such as 70% ethanol can also be used for CIP. The media also withstand the rigorous conditions used in process hygiene procedures such as sanitization. All strong oxidizing agents should, however, be avoided.

### Non-specific interaction

Studies (4) have demonstrated varying degrees of non-specific interaction between the media and acidic and basic proteins in the absence of salt. Such interactions are negligible in salt concentrations between 0.15–1.5 M NaCl.



**Fig. 7.** Performance of Superdex 200 prep grade measured as  $K_{av}$  values of a protein mixture after repeated treatment with (a) 0.1 M HCl and (b) 1 M NaOH. The separation medium was exposed to 1 M NaOH for repeated 8-hour periods at an ambient temperature of 22 °C. After each exposure period the  $K_{av}$  value was determined for a test mixture of proteins. Following an accumulated exposure time of 150 hours, the exposure periods were increased to 16 hours. Even after more than 300 hours accumulated exposure to NaOH,  $K_{av}$  values did not change significantly.

## Column characteristics

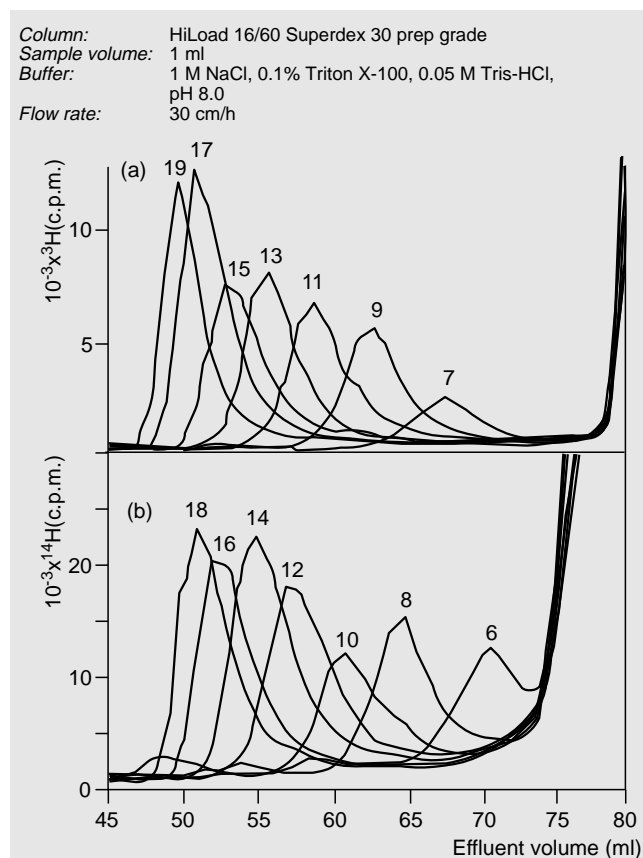
The XK columns into which the Superdex prep grade media are packed are modern, easy-to-use laboratory columns. Each has a precision bore borosilicate glass tube and a fitted thermostatic jacket. Dead volumes make up less than 0.1% of the total column volume, keeping sample dilution and band broadening to a minimum.

Flanged tubing and M6 connectors for connection to FPLC and BioPilot chromatography systems are supplied as standard. Accessory valves and connectors make even complex elutions easy to manage.

Every pre-packed HiLoad column is tested for number of theoretical plates per metre (N/m), asymmetry factor (Af) and bed height (mm). These stringent control measures ensure that HiLoad columns give reproducible results time after time.

## Applications

HiLoad Superdex 30 prep grade is optimized for proteins and peptides, but it can also be used with success to separate oligosaccharides. (Fig. 8.)



**Fig. 8.** Separation of oligosaccharides on Superdex 30 prep grade. The numbers above each peak indicate the number of monosaccharide units per molecule/chain. The oligosaccharides are applied one at a time. Each chromatogram represents seven superimposed analyses. (Reproduced by kind permission of Dr. Kerstin Lidholt, University of Uppsala, Uppsala, Sweden.)

HiLoad Superdex 75 prep grade separates proteins and peptides in the molecular weight range 3 000–70 000 daltons and performs best between 8 000 and 50 000, which is ideal for recombinant proteins. Figure 9 shows one example.

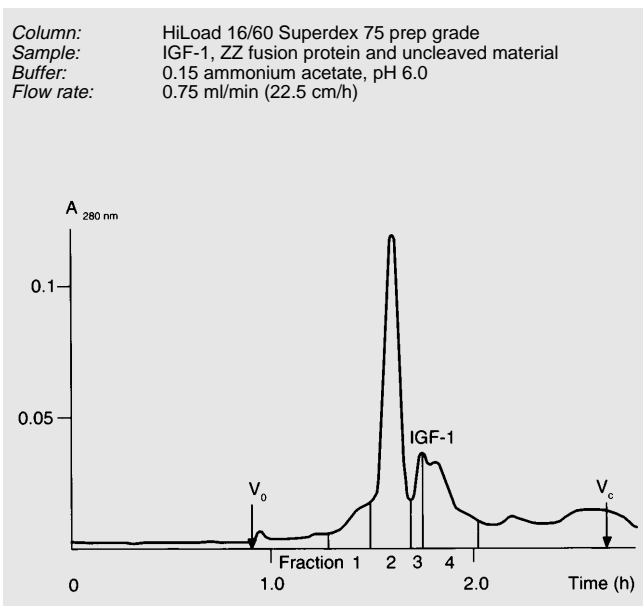


Fig. 9. Separation of recombinant IGF-1 (insulin-like growth factor 1, mol. wt. 7 600 daltons) from its fusion protein partner (ZZ, mol. wt. 14 500 daltons) and uncleaved material.

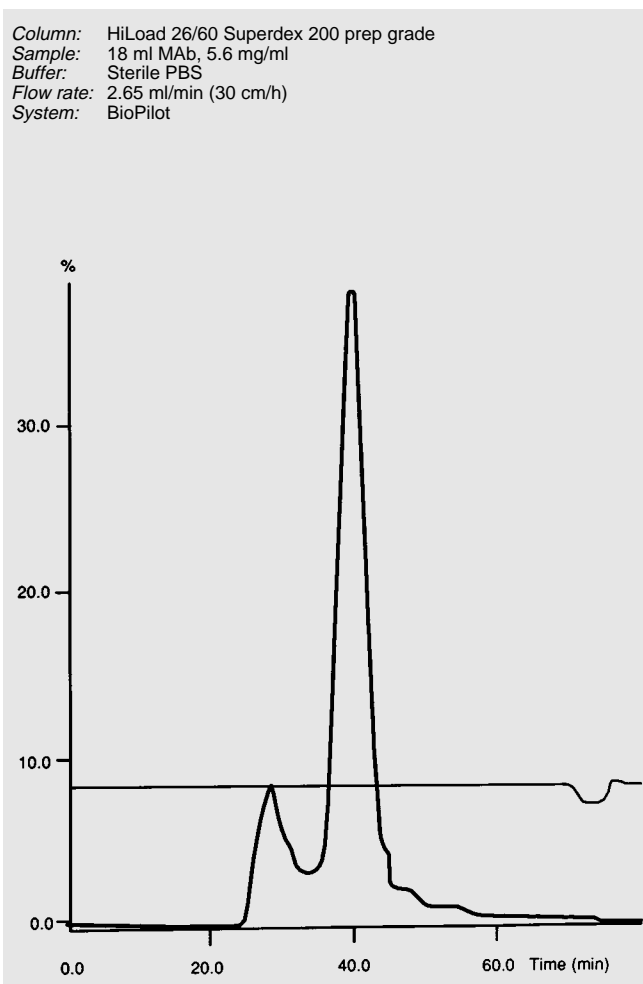


Fig. 10. Purification of mouse monoclonal IgG<sub>1</sub> anti-gp 120 for intravenous use in clinical studies on the clinical indication AIDS. (Purification developed in collaboration with B. Wahren *et al.* of the National Bacteriological Laboratory (SBL), Dept. of Virology, Sweden.)

HiLoad Superdex 200 prep grade has a total separation range of 10 000–600 000 daltons and separates with highest selectivity between 30 000 and 250 000. This grade separates monoclonal antibodies from critical contaminants and aggregates. Figure 10 shows an example.

## Ordering Information

Product	Code No.
HiLoad 16/60 Superdex 30 prep grade	17-1139-01
HiLoad 26/60 Superdex 30 prep grade	17-1140-01
HiLoad 16/60 Superdex 75 prep grade	17-1068-01
HiLoad 26/60 Superdex 75 prep grade	17-1070-01
HiLoad 16/60 Superdex 200 prep grade	17-1069-01
HiLoad 26/60 Superdex 200 prep grade	17-1071-01

## Packs of Superdex prep grade media

You can also order packs of Superdex 30, 75 and 200 prep grade media in the following sizes.

Product	Pack size	Code No.
Superdex 30 prep grade	150 ml	17-0905-01
Superdex 30 prep grade	1 litre	17-0905-03
Superdex 30 prep grade	5 litres	17-0905-04
Superdex 75 prep grade	150 ml	17-1044-01
Superdex 75 prep grade	1 litre	17-1044-02
Superdex 75 prep grade	5 litres	17-1044-04
Superdex 200 prep grade	150 ml	17-1043-01
Superdex 200 prep grade	1 litre	17-1043-02
Superdex 200 prep grade	5 litres	17-1043-04

## HiLoad columns for other separation techniques are:

### Ion exchange chromatography

Product	Code No.
HiLoad 16/10 Q Sepharose® High Performance	17-1064-01
HiLoad 26/10 Q Sepharose High Performance	17-1066-01
HiLoad 16/10 SP Sepharose High Performance	17-1137-01
HiLoad 26/10 SP Sepharose High Performance	17-1138-01

### Hydrophobic interaction chromatography

Product	Code No.
HiLoad 16/10 Phenyl Sepharose High Performance	17-1085-01
HiLoad 26/10 Phenyl Sepharose High Performance	17-1086-01

Laboratory and bulk packs of Sepharose High Performance media are also available.

## References

1. Chemical, physical and chromatographic properties of Superdex 75 prep grade and Superdex 200 prep grade gel filtration media. *J. Chromatogr.* 537 (1991) 17-32, Kägedal, L., *et al.*
2. Chemical properties of and solute-support interactions with the gel filtration medium Superdex 75 prep grade. *J. Chromatogr.* 514 (1990) 137-146, Johansson, B-L. *et al.*
3. Stability of Superdex 75 prep grade and Superdex 200 prep grade at different chromatographic conditions. *J. Chromatogr.* 547 (1991) 21-30, Drevin, I., Johansson, B-L.
4. Characterization of two new gel filtration media. I. Chromatographic and Physico-Chemical Properties. Eighth International Symposium on HPLC of Proteins, Peptides and Polynucleotides. Copenhagen Oct 31- Nov 2, 1988, poster 105 Engström, B., *et al.*
5. Data File "Superdex 30, 75 and 200 prep grade", Pharmacia BioProcess Technology, Code No. 18-1020-92.
6. Application Note 213 "Use of Superdex 30 prep grade in different application areas", Pharmacia BioProcess Technology.