Superdex prep grade and prepacked **HiLoad** columns

SIZE EXCLUSION CHROMATOGRAPHY

Superdex™ prep grade (pg) is a high resolution size exclusion chromatography resin (Fig 1). It is composed of cross-linked agarose and dextran. The steep selectivity of the dextran component and the high chemical and physical stability of the agarose give high-resolution separations at flow velocities up to 50 cm/h. Three resin types are available in laboratory and larger pack sizes: Superdex 30 prep grade, Superdex 75 prep grade, and Superdex 200 prep grade. Their main features are:

- Steep selectivity provides high resolution
- High chemical stability

The resins are also available in prepacked, high-performance HiLoad™ columns offered in two different column sizes, 16 and 26 mm diameter, both with 600 mm bed height (Fig 2). The HiLoad 16/600 and 26/600 columns provide a number of significant advantages for high resolution work:

- Prepacked for convenience and reproducibility
- High-resolution separation of biomolecules
- High chemical stability and easy scale-up
- Easy connection to, for example, ÄKTA™ chromatography systems

Each column is expertly packed and individually tested. This combination of prepacked convenience and reproducibility makes HiLoad Superdex pg columns a confident choice for fast, high-resolution size exclusion chromatography at preparative laboratory scale.

The columns run with a wide variety of equipment: ÄKTA systems or simple pump-based configurations.

Fig 1. Superdex size exclusion chromatography resins.

Fig 2. HiLoad Superdex 30, 75, and 200 pg columns bring convenience and high resolution to size exclusion chromatography. Each is available in two column sizes: HiLoad 16/600 and HiLoad 26/600.

Resin characteristics

Chemical stability

Superdex size exclusion chromatography resins are produced by the covalent binding of dextran to cross-linked, porous agarose particles.

Steep selectivity curves give exceptional resolution for biomolecules in the molecular weight range (M_r) up to 10 000 for Superdex 30 prep grade, M_r ~3000 to 70 000 for Superdex 75 prep grade, and M_r ~10 000 to 600 000 for Superdex 200 prep grade (Fig 3).

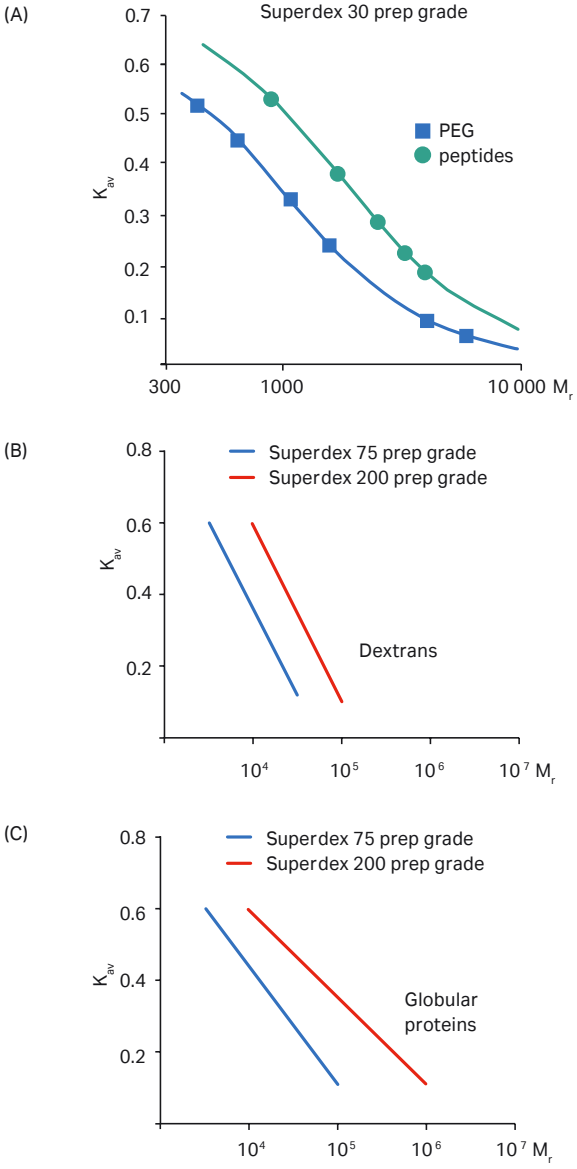

Fig 3. Selectivity curves from Superdex 30 prep grade, Superdex 75 prep grade, and Superdex 200 prep grade.

Moreover, the particle size of ~34 µm and narrow particle size distribution of Superdex prep grade resins give good separation performance without creating high backpressure.

Figures 4 and 5 show separations of different model proteins on HiLoad 16/600 Superdex 30 pg, 75 pg, and 200 pg.

Column: HiLoad 26/600 Superdex 30 pg
Sample: Mix of five synthetic peptides in 1% TFA
1: M_r 3894 2: M_r 3134 3: M_r 2365
4: M_r 1596 5: M_r 827
Sample volume: 50 µL
Buffer: 20 mM Tris-HCl, 0.25 M NaCl, pH 8.5
Flow rate: 1 mL/min (30 cm/h)

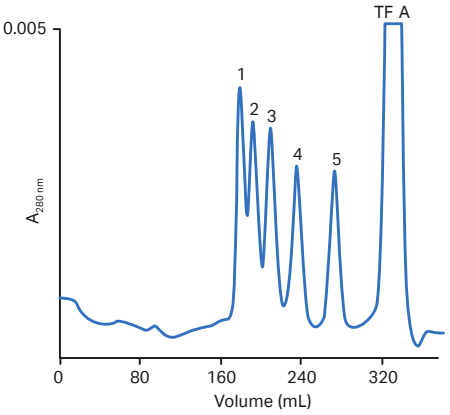

Fig 4. Separation of test substances on HiLoad 26/600 Superdex 30 pg. Superdex 30 prep grade resin is optimized for proteins/peptides below M_r 10 000.

Columns: (A) HiLoad 16/600 Superdex 75 pg
(B) HiLoad 16/600 Superdex 200 pg
Sample: 1. Myoglobulin, 1.5 mg/mL, M_r 17 000
2. Ovalbumin, 5 mg/mL, M_r 44 000
3. Albumin (human), 5 mg/mL, M_r 66 000
4. IgG, 0.2 mg/mL, M_r 158 000
5. Ferritin, 0.24 mg/mL, M_r 440 000
Sample volume: 500 µL
Buffer: 0.010 M phosphate buffer 0.14 M NaCl, 0.0027 M KCl, pH 7.4 (PBS)
Flow rate: 1 mL/min (30 cm/h)

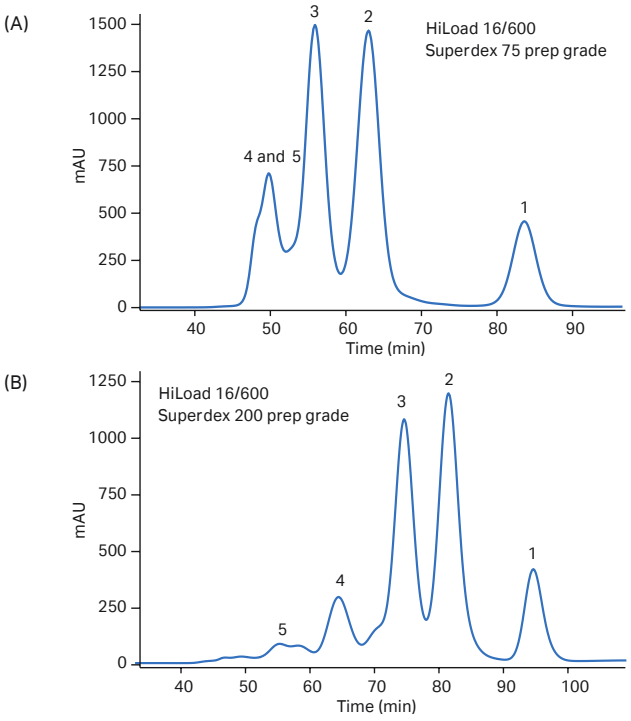

Fig 5. Comparison between the selectivity of Superdex 75 prep grade and Superdex 200 prep grade for model proteins. (A) Superdex 75 prep grade gives excellent resolution of the three proteins in the M_r range 17 000 to 67 000 while the two largest elute together in the void volume.
(B) Superdex 200 prep grade resolves these two largest proteins. The ferritin (5) contains aggregates and thus results in a double peak.

Table 1 summarizes the characteristics of the resins and columns. Superdex 30 prep grade, Superdex 75 prep grade, and Superdex 200 prep grade can be used in aqueous solutions over the pH range of 3 to 12 for continuous operation and over the pH range of 1 to 14 for cleaning-in-place (CIP). Chaotropic agents such as 6 M guanidine hydrochloride or 8 M urea, detergents (ionic and nonionic), and polar organic solvents can also be used for CIP.

Table 1. Characteristics of Superdex resin and prepacked HiLoad Superdex pg columns.

Matrix	Cross-linked agarose, spherical
Particle size, d_{50V} [1]	~34 µm
Fractionation range [M_r]	
Globular proteins	< 10 000 (Superdex 30 prep grade) ~3000 to 70 000 (Superdex 75 prep grade) ~10 000 to 600 000 (Superdex 200 prep grade)
Dextrans	~500 to 30 000 (Superdex 75 prep grade) ~1000 to 100 000 (Superdex 200 prep grade)
pH stability, operational[2]	3 to 12
pH stability, CIP[3]	1 to 14
Chemical stability	Stable to commonly used aqueous buffers 1 M acetic acid 0.01 M NaOH 8 M urea 6 M guanidine hydrochloride 30% isopropanol 30 acetonitrile 24% ethanol 0.001 M hydrochloric acid 1% SDS
Recommended operating flow velocity	10 to 50 cm/h[4]
Avoid	Strong oxidizing agents
Autoclavability	20 min at 121˚C, 1 cycle
Storage	
Superdex 30 prep grade	0.2 M sodium acetate in 20% ethanol, 4˚C to 30˚C
Superdex 75 prep grade	0.2 M sodium acetate in 20% ethanol, 4˚C to 30˚C
Superdex 200 prep grade	20% ethanol, 4˚C to 30˚C
Column volume	120 mL (HiLoad 16/600) 320 mL (HiLoad 26/600)
Sample volume	Up to 5 mL (HiLoad 16/600) Up to 13 mL (HiLoad 26/600)
Recommended operating flow rate[5]	0.3 to 1.6 mL/min for HiLoad 16/600 0.9 to 4.4 mL/min for HiLoad 26/600
Theoretical plates	>13 000 m-1
Maximum operating pressure	0.3 MPa (3 bar, 42 psi)
HiLoad column hardware pressure limit	0.5 MPa (5 bar, 73 psi)
Column fittings	1/16" (Valco™)

[1] Median particle size of the cumulative volume distribution

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

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

[4] 20 cm diameter, 83 cm bed height, at room temperature using buffers with the same viscosity as water

[5] At room temperature in H_2O

Figure 6 illustrates the stability of Superdex 200 prep grade in 0.1 M HCl and 1.0 M NaOH, a feature that is important for CIP procedures.

The resins also withstand the rigorous conditions used in process hygiene procedures such as sanitization. All strong oxidizing agents should, however, be avoided.

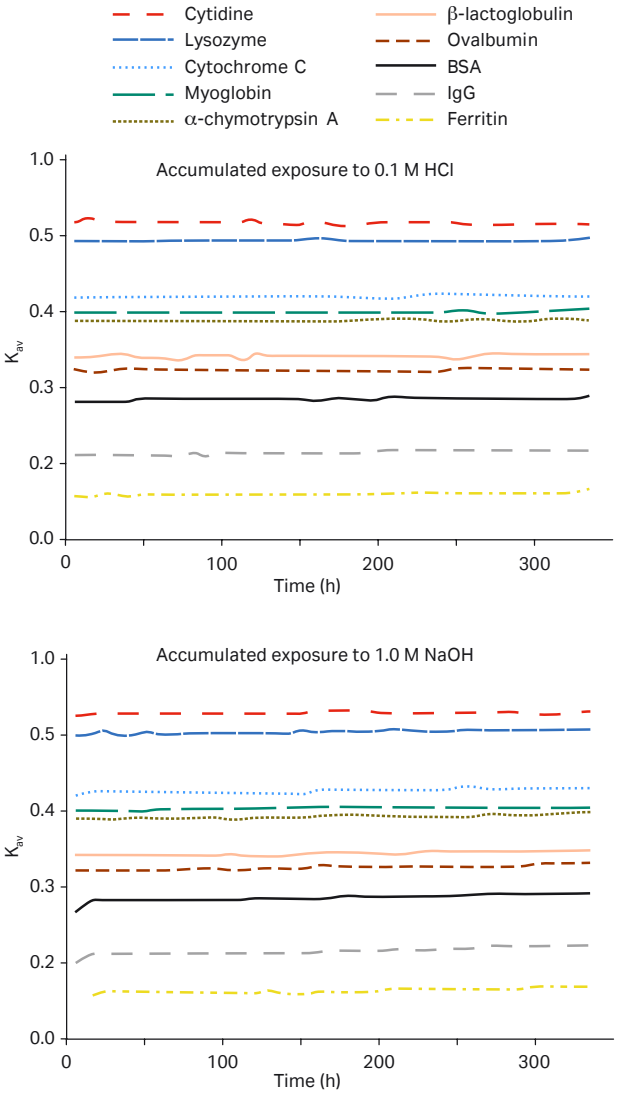

Fig 6. Performance of Superdex 200 prep grade measured as Kav values of a protein mixture after repeated treatment with (A) 0.1 M HCl or (B) 1 M NaOH. The resin was exposed for repeated 8 h periods at a temperature of 22°C. After each exposure period the K_{av} value was determined for a test mixture of proteins. Following an accumulated exposure time of 150 h, the exposure periods were increased to 16 h. Even after more than 300 h accumulated exposure, K_{av} values did not change significantly.

Nonspecific interaction

Studies have demonstrated varying degrees of nonspecific interaction between the resins and acidic as well as basic proteins in the absence of salt. Such interactions are negligible in salt concentrations between 0.15 and 1.5 M NaCl.

Column characteristics

HiLoad columns are easy-to-use laboratory columns prepacked with Superdex prep grade resins. 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.

Valco fittings (1/16") are standard and provide easy and direct connection to ÄKTA systems.

Every prepacked HiLoad column is tested for number of theoretical plates per meter (N/m), asymmetry factor (Af), and bed height (mm). This stringent control helps to secure reproducible results time after time.

Operation

Optimization

Size exclusion chromatography is widely used in chromatography, particularly for polishing of the final product. In addition to removal of product aggregates, the technique also allows the transfer of product to formulation buffer.

When optimizing a size exclusion chromatography step to achieve maximum productivity, the following parameters need careful consideration:

a) feed concentration

b) flow velocity

c) feed volume

Resin: Superdex 30 prep grade
Column: Tricorn™ 10/300
Feed material: Insulin-like growth factor (IGF-1) containing monomers, dimers, and multimers
Feed volume: 0.8% V_t
Buffer: 0.05 sodium acetate, 0.1 M sodium chloride, pH 5.0
Flow velocity: 30 cm/h

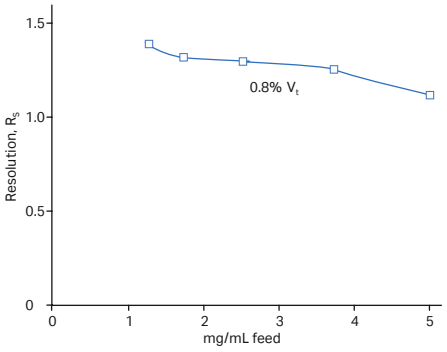

Fig 7A. Influence of feed concentration on the resolution of IGF-1 and multimers on Superdex 30 prep grade.

Resin: Superdex 30 prep grade
Column: Tricorn 10/300
Feed material: IGF-1 containing monomers and dimers
Feed concentration: a: 1.25 mg/mL, b: 5 mg/mL
Feed volume: 0.8% V_t
Buffer: 0.05 sodium acetate, 0.1 M sodium chloride, pH 5.0

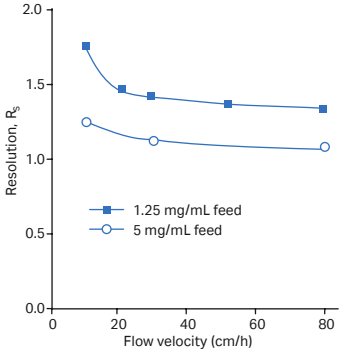

Fig 7B. Influence of flow velocity on the resolution of IGF-1 and multimers on Superdex 30 prep grade.

Resin: Superdex 30 prep grade
Column: Tricorn 10/300
Feed material: IGF-1 containing monomers, dimers, and multimers
Feed concentration: 2.5 mg/mL
Buffer: 0.05 sodium acetate, 0.1 M sodium chloride, pH 5.0
Flow velocity: 30 cm/h

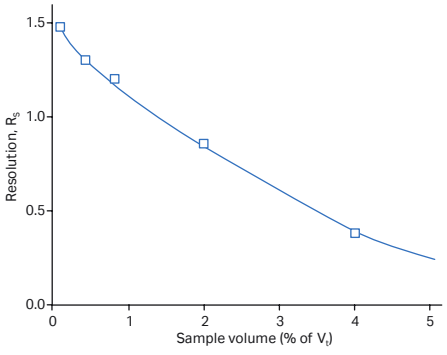

Fig 7C. Influence of feed volume on the resolution of IGF-1 and multimers on Superdex 30 prep grade.

Resin: Superdex 200 prep grade
Column: XK 16/70
Feed material: Solution of transferrin (M_r 81 000) and IgG (M_r 160 000) by equal weight
Feed volume: 0.8% V_t
Buffer: 0.05 sodium phosphate, 0.1 M sodium chloride, pH 7.2
Flow velocity: 30 cm/h

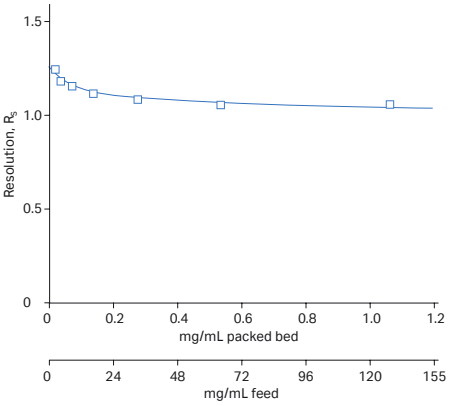

Fig 7D. Influence of feed concentration on the resolution of transferrin and IgG on Superdex 200 prep grade.

Resin: Superdex 200 prep grade
Column: XK 16/70
Feed material: Solution of transferrin (M_r 81 000) and IgG (M_r 160 000) by equal weight
Feed concentration: a) 8 mg/mL, b) 64 mg/mL
Feed volume: 0.8% V_t
Buffer: 0.05 sodium acetate, pH 5.0 0.2 M sodium phosphate, 0.1 M sodium chloride, pH 7.2

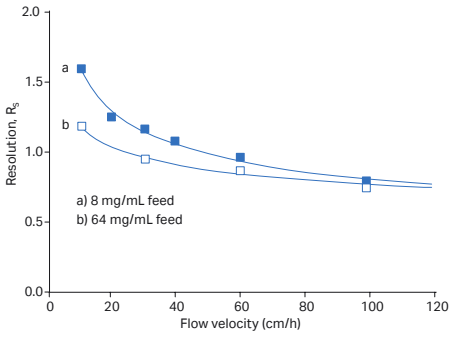

Fig 7E. Influence of flow velocity on the resolution of transferrin and IgG on Superdex 200 prep grade.

Resin: Superdex 200 prep grade
Column: XK 16/70
Feed material: Solution of transferrin (M_r 81 000) and IgG (M_r 160 000) by equal weight
Feed concentration: 8 mg/mL
Buffer: 0.02 sodium phosphate, 0.1 M sodium chloride, pH 7.2
Flow velocity: 30 cm/h

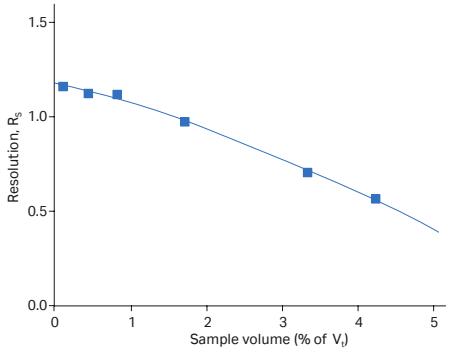

Fig 7F. Influence of feed volume on the resolution of transferrin and IgG on Superdex 200 prep grade.

The good flow properties and steep selectivity of Superdex prep grade resins allow separation conditions to be optimized for maximum productivity. However, in any chromatographic process there is always a balance between resolution and productivity. Figures 7 A to F show the influence of feed concentration, flow velocity, and feed volume on resolution on Superdex prep grade. Figures 7 A to C pertain to Superdex 30 prep grade and Figures 7 D to F pertain to Superdex 200 prep grade.

To illustrate how feed concentration, flow velocity, and/or feed volume influence the balance between resolution and productivity, IgG and transferrin were separated on Superdex 200 prep grade (Fig 7 D). The example shows that a feed concentration in the range 24 to 155 mg/mL does not affect resolution. High sample concentration does, however, reduce resolution, but this effect is less at higher flow velocities (Fig 7 E). Feed volume influences resolution the most (Fig 7 F). From the results it can be seen that it is advantageous to use high feed concentration, a high flow velocity, and to adjust feed volume to obtain the required resolution. Each case, however, has to be optimized individually.

Applications

HiLoad Superdex 30 pg

HiLoad Superdex 30 pg is optimized for proteins and peptides (Fig 8), but it can also be used with success to separate oligosaccharides (Fig 9).

Column:	HiLoad 16/600 Superdex 30 pg (120 mL)
Sample:	Tryptic digest of human inter-α-inhibitor
Sample volume:	1 mL
Buffer:	PBS, 100 mM NaCl, pH 7.4
Flow rate:	1 mL/min (30 cm/h)
Detection:	UV, absorbance 220 nm

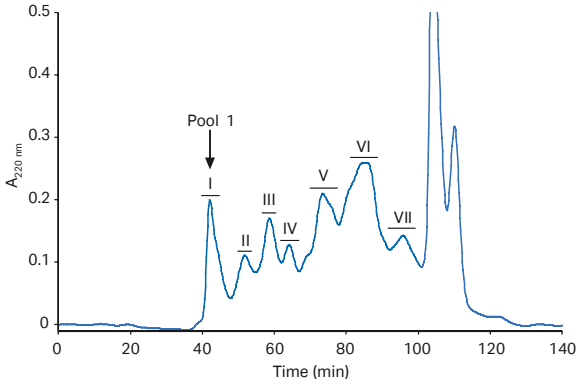

Fig 8. Purification of tryptic digest of human inter-α-inhibitor using HiLoad 16/600 Superdex 30 pg resulted in seven separated peaks (I–VII). Pool 1 contains the large peptide fragment including the intact carbohydrate cross-link. The size of this fragment is mainly due to the large hydrodynamic volume of glycosaminoglycan (GAG). Size exclusion chromatography is a good choice for isolating peptides containing large carbohydrate moieties. Reproduced by kind permission of Dr. J. Enghild, Dept. of Molecular and Structural Biology, University of Aarhus, Denmark.

Column:	HiLoad 16/600 Superdex 30 pg (120 mL)
Sample volume:	1 mL
Buffer:	0.05 M Tris-HCl, 1 M NaCl, 0.1% Triton™ X-100, pH 8.0
Flow rate:	1 mL/min (30 cm/h)

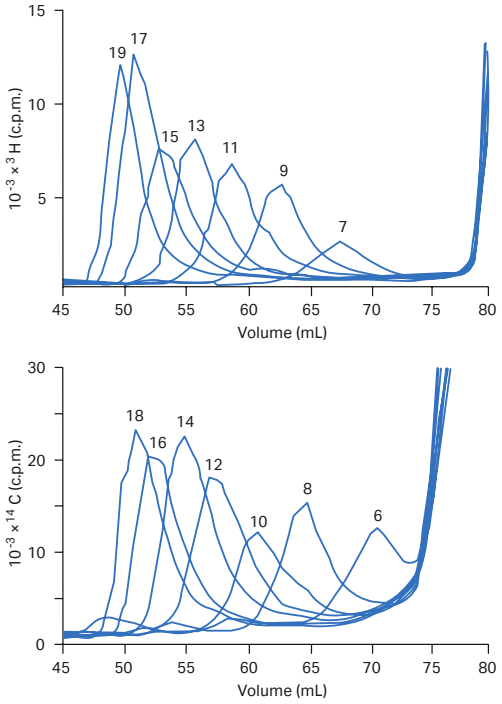

Fig 9. Separation of oligosaccharides on HiLoad 16/600 Superdex 30 pg. The numbers above each peak indicate the number of monosaccharide units per molecule/chain. The oligosaccharides are applied one at a time. Each chromatogram represents seven superimposed analyses. Reproduced by kind permission of Dr. K. Lidholt, University of Uppsala, Sweden.

HiLoad Superdex 75 pg

HiLoad Superdex 75 pg separates proteins and peptides in the molecular weight range M_r ~3000 to 70 000 and performs best between M_r ~8000 and 50 000 (Fig 10 and 11).

Column: HiLoad 16/600 Superdex 75 pg (120 mL)
Sample: IGF-1, ZZ fusion protein, and uncleaved material
Buffer: 0.15 ammonium acetate, pH 6.0
Flow rate: 0.75 mL/min (22.5 cm/h)

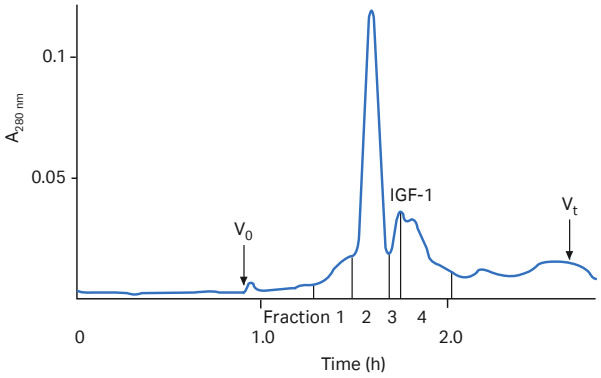

Fig 10. Separation of recombinant IGF-1 (M_r 7600) from its ZZ fusion protein partner (M_r 14 500) and uncleaved material. V_0 = column void volume. V_t = total column volume.

Column: HiLoad 16/600 Superdex 75 pg (120 mL)
Sample: endo-β-1,4-glucanase (cellulase) from blue mussel *(Mytilusedilus)*. Concentrated, eluted endoglucanase active material from an affinity step
Sample volume: 2 mL
Buffer: 20 mM sodium phosphate buffer, 0.3 M NaCl, pH 7.0
Flow rate: 1 mL/min (30 cm/h)

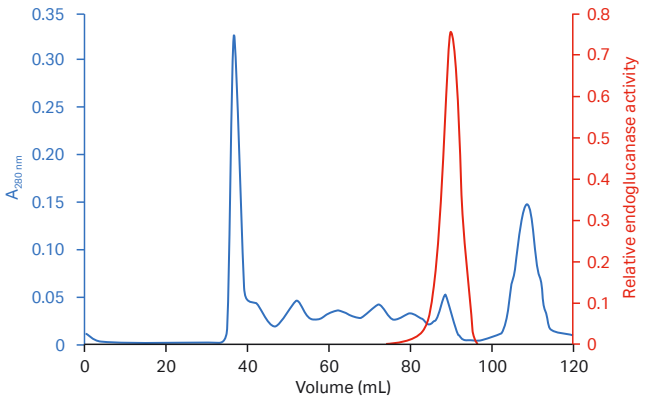

Fig 11. Intermediate purification step in laboratory scale on HiLoad 16/60 Superdex 75 pg. Concentrated sample (2 mL) of the endoglucanase active material eluted from an affinity step was applied on the column. Reproduced by kind permission of Dr. B. Xu, University of Uppsala, Sweden.

HiLoad Superdex 200 pg

HiLoad Superdex 200 pg has a fractionation range of M_r ~10 000 to 600 000 and separates with highest selectivity between M_r ~30 000 and 250 000. Superdex 200 separates monoclonal antibodies from critical contaminants and aggregates (Fig 12).

Figure 13 shows the purification of mouse monoclonal IgG_{2b} directly from cell supernatant. The reproducibility when scaling up from a HiLoad 16/600 to a HiLoad 26/600 column is also shown.

Column: (A) HiLoad 16/600 Superdex 200 pg
(B) HiLoad 26/600 Superdex 200 pg
Sample: Monoclonal antibodies purified on HiScreen™ MabSelect SuRe™ LX
Sample volume: (A) 1 mL
(B) 3 mL
Buffer: 0.010 M phosphate buffer, 0.14 M NaCl, 0.0027 M KCl, pH 7.4 (PBS)
Flow rate: (A) 1 mL/min (30 cm/h)
(B) 2.5 mL/min (28 cm/h)

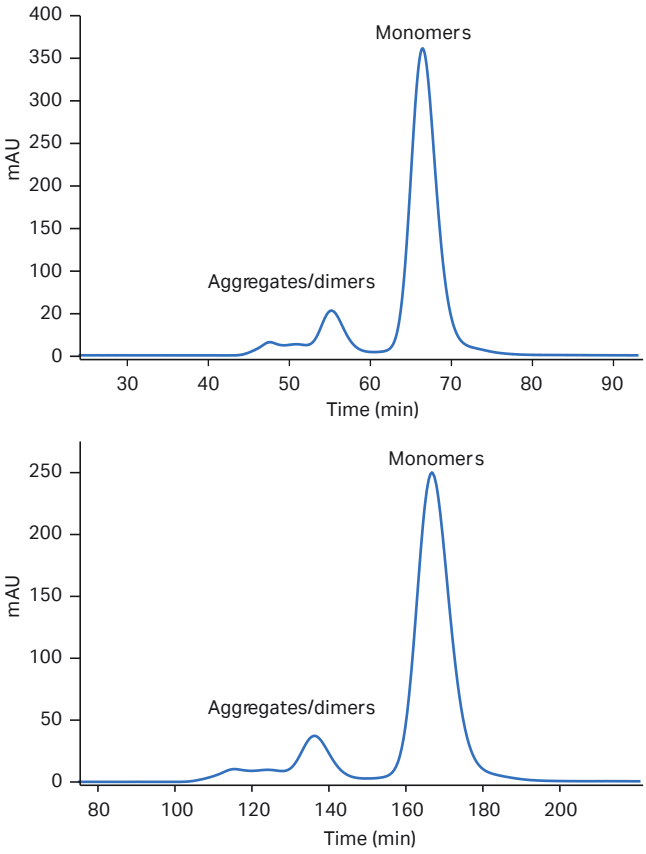

Fig 12. Separation of monoclonal antibody monomers from aggregates/dimers on HiLoad 16/600 Superdex 200 pg and HiLoad 26/600 Superdex 200 pg. 85% of IgG4 was monomers (9.5 mg).

Columns:	HiLoad Superdex 200 pg
Column volumes, V_t:	(A) 120 mL (16/600) (B) 320 mL (26/600)
Sample:	Mouse monoclonal cell supernatant, IgG_{2b} incl. 1% fetal calf serum
Sample pretreatment:	Concentration ≈ 40× in concentration cell
Sample volume:	(A) 1.2 mL (B) 3.2 mL
Buffer:	50 mM NaH_2PO_4, 0.15 M NaCl, pH 7.0
Flow rates:	(A) 1.6 mL/min (50 cm/h) (B) 4.4 mL/min (50 cm/h) (max recommended flow rates)

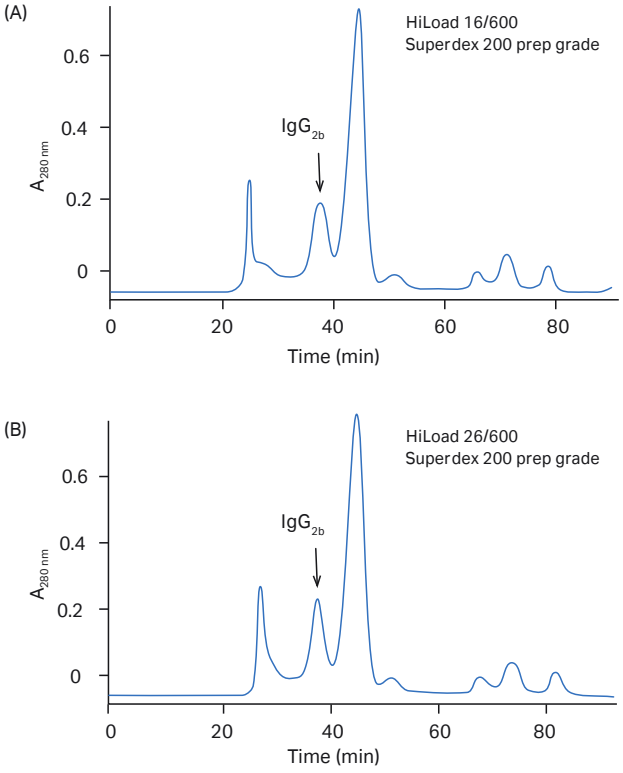

Fig 13. Purification of mouse monoclonal IgG2b from cell supernatant using (A) HiLoad 16/600 Superdex 200 pg, column volume 120 mL or (B) HiLoad 26/600 Superdex 200 pg, column volume 320 mL. Almost identical separations are the result, even when using prepacked columns of different sizes.

Cleaning-in-place

The chemical stability of Superdex prep grade resins permits the use of effective CIP protocols that help to ensure a longer column life and good process economy. Repeated separation cycles tend to cause a build-up of contaminants and specific CIP protocols should therefore be developed as part of the routine separation process.

Table 2 gives two examples of effective CIP protocols.

Sanitization

Sanitization is the use of chemical agents to inactivate microbial contaminants in the form of vegetative cells. It also helps maintain a high level of process hygiene and process economy.

An example of an effective sanitization protocol is given in Table 2.

Table 2. CIP and sanitization protocols

Purpose	Procedure
To remove hydrophobic proteins or lipoproteins	Wash the column with one column volume (CV) of 0.5 M NaOH at 20 cm/h, with reversed direction of flow
To remove lipid and very hydrophobic proteins	Wash the column with two CV of 30% isopropanol at 10 cm/h, with reversed direction of flow
Sanitization	Expose the column to 0.5 M NaOH for 30 to 60 min at room temperature

Note! After treatment with sodium hydroxide, isopropanol, or acetonitrile, wash the column with water prior to re-equilibration with buffer

Storage

Storage conditions for the different Superdex prep grade resins are listed in Table 3.

Table 3. Storage conditions for Superdex prep grade resins

Resin	Storage
Superdex 30 prep grade	0.2 M sodium acetate in 20% ethanol, 4°C to 30°C
Superdex 75 prep grade	0.2 M sodium acetate in 20% ethanol, 4°C to 30°C
Superdex 200 prep grade	20% ethanol, 4°C to 30°C

Ordering information

Product, resin	Quantity	Product code
Superdex 30 prep grade	150 mL	17090501
Superdex 30 prep grade	1 L	17090503
Superdex 30 prep grade	5 L	17090504
Superdex 75 prep grade	150 mL	17104401
Superdex 75 prep grade	1 L	17104402
Superdex 75 prep grade	5 L	17104404
Superdex 200 prep grade	150 mL	17104301
Superdex 200 prep grade	1 L	17104302
Superdex 200 prep grade	5 L	17104304
Superdex 200 prep grade	10 L	17104305

Product, prepacked column	Quantity	Product code
HiLoad 16/600 Superdex 30 pg	1 × 120 mL	28989331
HiLoad 26/600 Superdex 30 pg	1 × 320 mL	28989332
HiLoad 16/600 Superdex 75 pg	1 × 120 mL	28989333
HiLoad 26/600 Superdex 75 pg	1 × 320 mL	28989334
HiLoad 16/600 Superdex 200 pg	1 × 120 mL	28989335
HiLoad 26/600 Superdex 200 pg	1 × 320 mL	28989336

Accessories	No. supplied	Product code
Accessory kit XK 16*		28989978
Accessory kit XK 26*		28989979
Support screen XK 16	5	19065101
Support screen XK 26	5	18937701
Net ring (10 µm) XK 16	5	18876101
Net ring (10 µm) XK 26	5	18876001
O-ring XK 16	5	19016301
O-ring XK 26	5	28978227
Stop Plug Female, 1/16"	5	11000464
Tricorn Storage/Shipping Device	1	18117643

* 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 system, and 1 tool for dismantling

Related literature	Product code
Size Exclusion Chromatography: Principles and Methods	18102218
Size Exclusion Chromatography Columns and Media, Selection Guide	18112419
Prepacked chromatography columns for ÄKTA systems, Selection Guide	28931778

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