GE Healthcare
Life Sciences

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

HiLoad columns are XK laboratory columns prepacked with Superdex prep grade for gel filtration. Superdex prep grade is a 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 work:

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

Three types of Superdex prep grade are available in prepacked HiLoad columns: Superdex 30 prep grade, Superdex 75 prep grade, and Superdex 200 prep grade. All are prepacked to 600 mm bed heights in 16 mm or 26 mm diameter columns.

Each column is expertly packed and individually tested. This combination of prepacked convenience and reproducibility make HiLoad Superdex prep grade columns a confident choice for fast, high-resolution gel filtration at preparative scale.

The columns run with a wide variety of equipment, including simple pump-based configurations and ÄKTA design systems. Superdex gel filtration media are produced by the covalent binding of dextran to highly cross-linked porous agarose beads.

Fig 1. HiLoad Superdex 30, 75, and 200 prep grade columns bring convenience and high resolution to gel filtration. Each is available in two column sizes, HiLoad 16/600 and HiLoad 26/600.

Steep selectivity curves give unmatched resolution for biomolecules in the molecular weight range up to 10 000 for Superdex 30 prep grade (Fig 1), 3000 to 70 000 for Superdex 75 prep grade, and 10 000 to 600 000 for Superdex 200 prep grade (Fig 2).

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 backpressure.

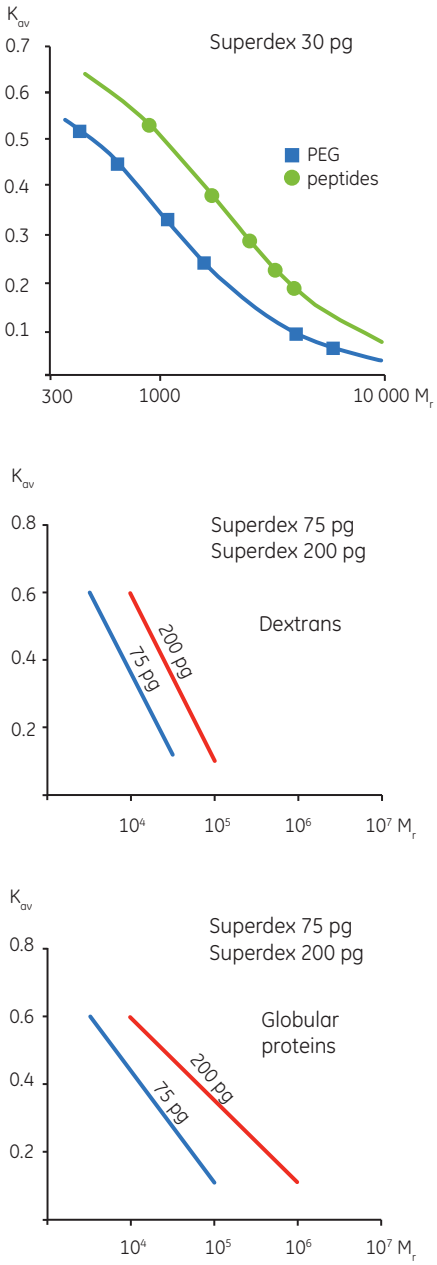

Fig 2. Selectivity curves from Superdex 30 pg, Superdex 75 pg, and Superdex 200 pg.

Figures 3 and 4 show separations of different model proteins on HiLoad 16/600 Superdex 30 pg, 75 pg, and 200 pg.

Table 1 summarizes the characteristics of the media and columns.

Column: HiLoad 26/600 Superdex 30 prep grade
Sample: 50 µl mix of five synthetic peptides in 1% TFA
1: M_r 3 894
2: M_r 3 134
3: M_r 2 365
4: M_r 1 596
5: M_r 827
Buffer: 20 mM Tris-HCl, 0.25 M NaCl, pH 8.5
Flow rate: 1 ml/min (30 cm/h)

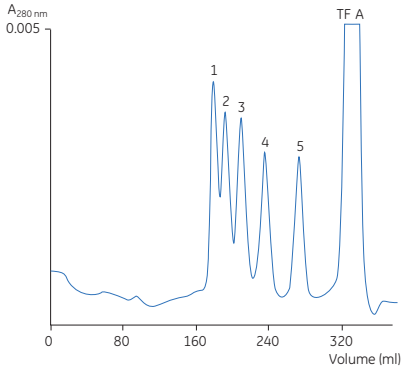

Fig 3. Separation of test substances on HiLoad 26/600 Superdex 30 prep grade. Superdex 30 prep grade is optimized for proteins/peptides below M_r 10 000.

Columns: A) HiLoad 16/600 Superdex 75 prep grade
B) HiLoad 16/600 Superdex 200 prep grade
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)

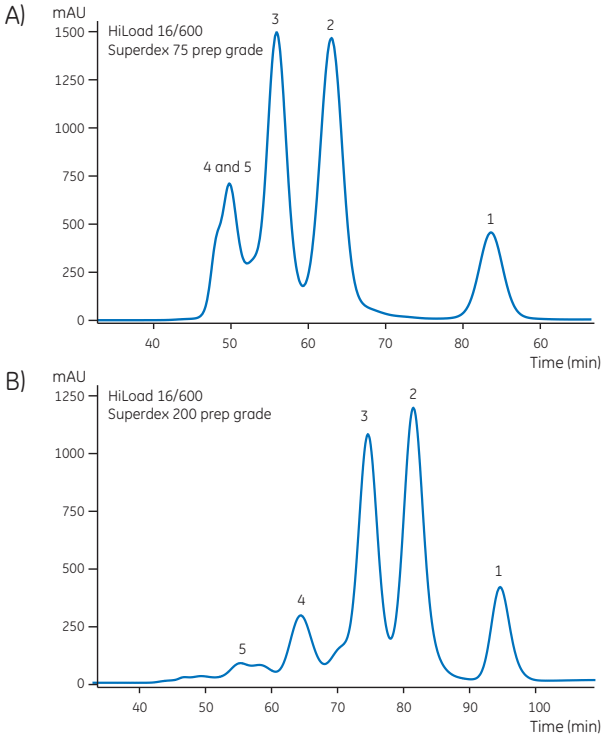

Fig 4. Comparison of the selectivity of Superdex 75 prep grade and Superdex 200 prep grade for model proteins. Superdex 75 prep grade (A) gives excellent resolution of the three proteins in the M_r range 17 000 to 67 000 while the two largest elute together in the void volume. Superdex 200 prep grade (B) resolves these two largest proteins. The ferritin (5) contains aggregates and thus results in a double peak.

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

Matrix	Dextran covalently bound to highly cross-linked agarose
Average particle size	34 µm
Separation range (M_r)	< 10 000 (Superdex 30 pg)
globular proteins	3×10^3–7×10^4 (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	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 flow rate	10–50 cm/h at room temperature (0.3–1.6 ml/min for HiLoad 16/600, 0.9–4.4 ml/min for HiLoad 26/600)
Theoretical plates	>13 000 m^{-1}
Maximum pressure over the packed bed during operation, Δp[1]	0.3 MPa, 3 bar, 42 psi
HiLoad column hardware pressure limit	0.5 MPa, 5 bar, 73 psi
Column fittings	1/16" (valco)
pH stability	
long term and working range	3 to 12
short term	1 to 14
Solutions in which the media is stable	All commonly used aqueous buffers, pH 3–12 1 M acetic acid 1 M sodium hydroxide 8 M urea 6 M guanidine hydrochloride 30% isopropanol 30% acetonitrile 70% ethanol
Storage	
Superdex 30 prep grade	0.2 M sodium acetate in 20% ethanol at 4°C to 30°C
Superdex 75 prep grade	0.2 M sodium acetate in 20% ethanol at 4°C to 30°C
Superdex 200 prep grade	20% ethanol at 4°C to 30°C

[1] H_2O at room temperature

Chromatography media characteristics

Chemical stability

Chromatography media based on highly cross-linked agarose are very stable. Low sample volumes give high resolution even at high flow rates.

Superdex 30 prep grade, Superdex 75 prep grade, and Superdex 200 prep grade may be used in aqueous solutions over the range pH 3 to 12 for continuous operation and over the range pH 1 to 14 for cleaning-in-place (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.

Extensive studies, including long-term exposure to harsh chemical treatment, have demonstrated that Superdex prep grade has extremely high chemical stability. Figure 5 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 CIP procedures.

Nonspecific interaction

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

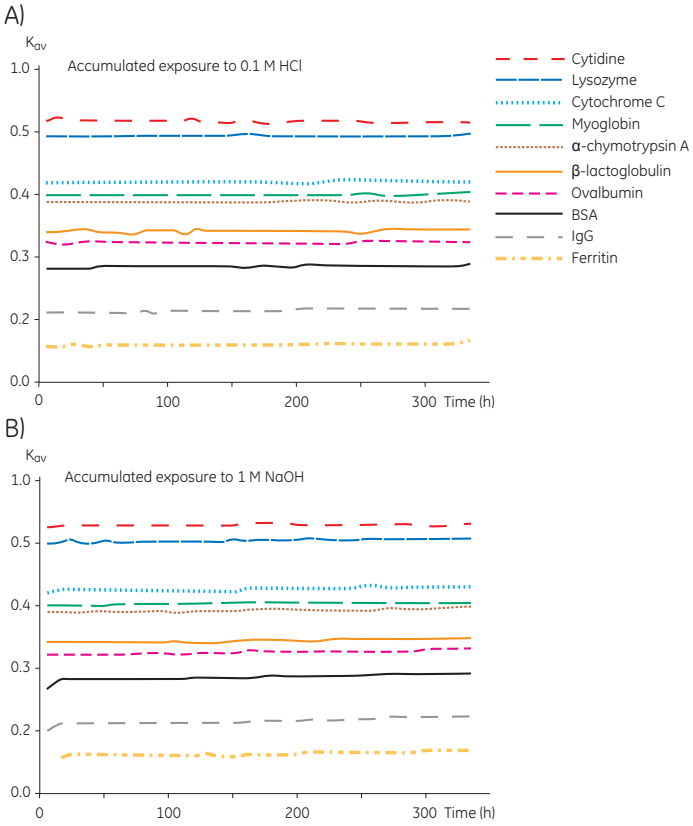

Fig 5. Performance of Superdex 200 prep grade measured as K_{av} values of a protein mixture after repeated treatment with (A) 0.1 M HCl and (B) 1 M NaOH. The chromatography medium was exposed for repeated 8 h periods at temperature of 22°C. After each exposure period the K_{av} value was determined 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.

Column characteristics

The XK columns prepacked with Superdex prep grade media are 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.

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

Every prepacked HiLoad column is tested for number of theoretical plates per meter (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

HiLoad Superdex 30 prep grade is optimized for proteins and peptides (Fig 7), but it can also be used with success to separate oligosaccharides (Fig 6).

Column: HiLoad 16/600 Superdex 30 prep grade (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)

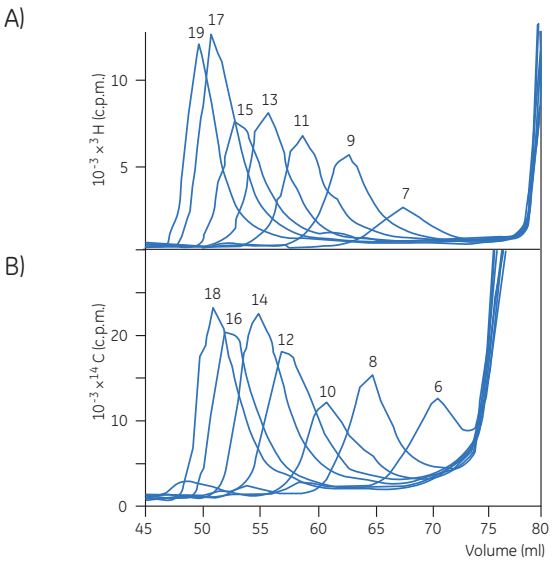

Fig 6. Separation of oligosaccharides on HiLoad 16/600 Superdex 30 prep grade. The numbers above each peak indicate the number of monosaccharide units per molecule/chain. The oligosaccharides are applied one at a time. Each chromatogram represents seven superimposed analyses. Reproduced by kind permission of Dr. K. Lidholt, University of Uppsala, Sweden.

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

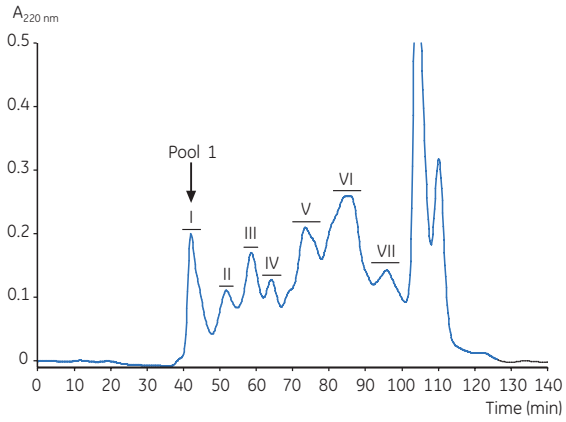

Fig 7. Purification of tryptic digest of human inter-α-inhibitor using HiLoad 16/600 Superdex 30 prep grade resulted in seven separated peaks (I–VII). Pool I contains the large peptide fragment including the intact carbohydrate cross-link. The size of this fragment is mainly due to the large hydrodynamic volume of the GAG (glycosaminoglycan). Gel filtration 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.

HiLoad Superdex 75 prep grade

HiLoad Superdex 75 prep grade separates proteins and peptides in the molecular weight range, M_r 3000–70 000 and performs best between 8000 and 50 000 (Figs 8 and 9).

Column: HiLoad 16/600 Superdex 75 prep grade (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)

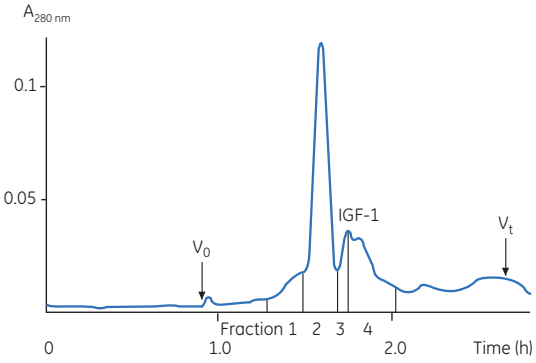

Fig 8. Separation of recombinant IGF-1 (insulin-like growth factor 1, M_r 7600) from its fusion protein partner (ZZ, M_r 14 500) and uncleaved material. V_0= Column void volume. V_t= Total column volume.

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

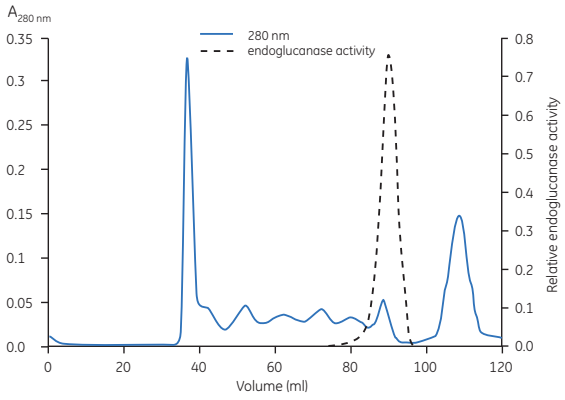

Fig 9. Intermediate purification step in lab scale on HiLoad 16/60 Superdex 75 prep grade. Two ml concentrated sample of the endoglucanase active material eluted from an affinity step was applied on the column. Reproduced by kind permission of Dr. B. Xu, University of Uppsala, Sweden.

HiLoad Superdex 200 prep grade

Figure 10 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.

HiLoad Superdex 200 prep grade has a separation range of M_r 10 000–600 000 and separates with highest selectivity between 30 000 and 250 000. Superdex 200 separates monoclonal antibodies from critical contaminants and aggregates (e.g., Fig 11).

Columns: HiLoad Superdex 200 prep grade
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 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)

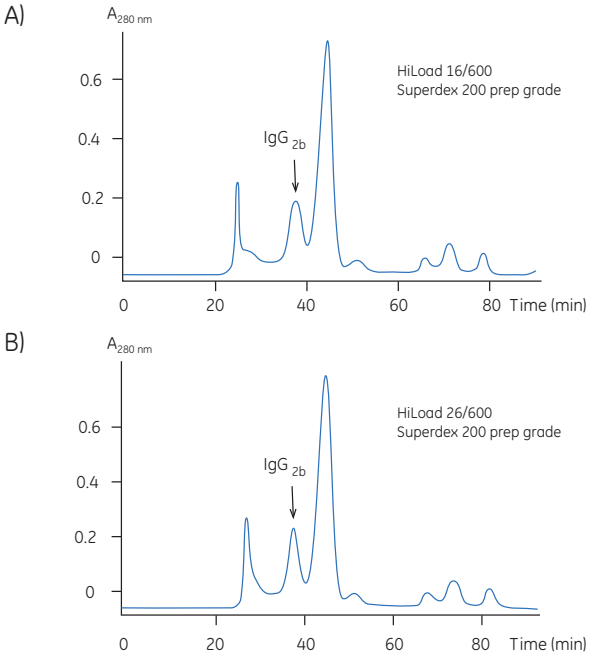

Fig 10. Purification of mouse monoclonal IgG_{2b} from cell supernatant using A) HiLoad 16/600 Superdex 200 prep grade, column volume 120 ml and B) HiLoad 26/600 Superdex 200 prep grade, column volume 320 ml. Almost identical separations are the result, even using prepacked columns of different sizes.

A)
Column: HiLoad 16/600 Superdex 200 prep grade
Sample: Monoclonal antibodies purified on HiScreen™ MabSelect SuRe™ LX
Sample volume: 1 ml
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)

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

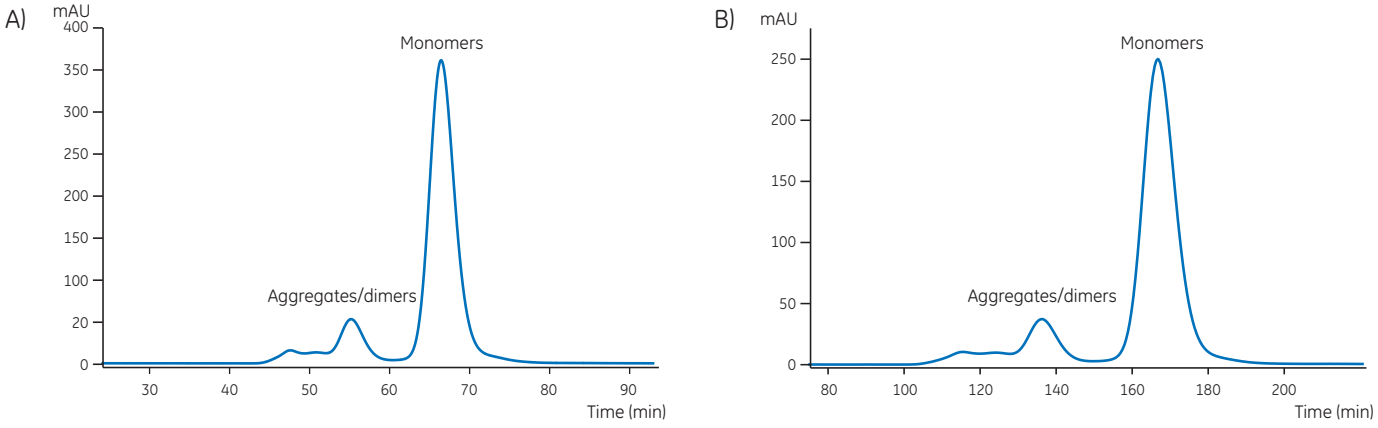

Fig 11. Separation of monoclonal antibody monomers from aggregates/dimers on HiLoad 16/600 Superdex 200 prep grade and HiLoad 26/600 Superdex 200 prep grade. 85% of IgG_4 was monomers (9.5 mg).

Ordering Information

Product	Quantity	Code no.
HiLoad 16/600 Superdex 30 prep grade	1 × 120 ml	28-9893-31
HiLoad 26/600 Superdex 30 prep grade	1 × 320 ml	28-9893-32
HiLoad 16/600 Superdex 75 prep grade	1 × 120 ml	28-9893-33
HiLoad 26/600 Superdex 75 prep grade	1 × 320 ml	28-9893-34
HiLoad 16/600 Superdex 200 prep grade	1 × 120 ml	28-9893-35
HiLoad 26/600 Superdex 200 prep grade	1 × 320 ml	28-9893-36

Accessories	No. supplied	Code no.
Accessory kit XK 16*		28-9899-78
Accessory kit XK 26*		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 products	Quantity	Code no.
Superdex Peptide PC 3.2/30	1 × 2.4 ml	17-1458-01
Superdex Peptide 10/300 GL	1 × 24 ml	17-5176-01
Superdex 30 prep grade	150 ml	17-0905-01
Superdex 75 PC 3.2/30	1 × 2.4 ml	17-0771-01
Superdex 75 5/150 GL	1 × 3 ml	28-9205-04
Superdex 75 10/300 GL	1 × 24 ml	17-5174-01
Superdex 75 prep grade	150 ml	17-1044-01
Superdex 200 PC 3.2/30	1 × 2.4 ml	17-1089-01
Superdex 200 5/150 GL	1 × 3 ml	28-9065-61
Superdex 200 10/300 GL	1 × 24 ml	17-5175-01
Superdex 200 prep grade	150 ml	17-1043-01

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

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