Superdex High-performance Columns

GEL FILTRATION

Superdex™ prepacked columns (Fig 1) are designed for high-performance, laboratory-scale separations of proteins, peptides, and other biomolecules according to size. High-performance gel filtration offers fast separations with high resolution for a variety of applications including protein purification, studies of complex formation, and screening of uncharacterized samples.

The columns offer:

- High-resolution separations of proteins, peptides, and other biomolecules according to size
- Fast gel filtration
- Separations in three molecular weight ranges from 100 to 600 000 M_r
- Excellent reproducibility and durability
- Simple scale-up

Gel filtration

Gel filtration separates molecules according to differences in size as they pass through a gel filtration medium packed in a column. Unlike ion exchange or affinity chromatography, molecules do not bind to the chromatography medium so buffer composition does not directly affect resolution (the degree of separation between peaks). Consequently, a significant advantage of gel filtration is that conditions can be varied to suit the type of sample or the requirements for further purification, analysis, or storage without altering the separation.

Gel filtration is an excellent technique for discriminating monomer, oligomer, or aggregated forms of a target protein.

Fig 1. Superdex 5/150 GL and 10/300 GL prepacked Tricorn™ columns.

Media characteristics

Superdex prepacked columns for gel filtration are available with three different media: Superdex Peptide, Superdex 75, and Superdex 200. Table 1 summarizes the main characteristics of Superdex media.

High-resolution separations of biomolecules according to size

Superdex has a special composite matrix of dextran and agarose (Fig 2). The matrix combines the excellent gel filtration properties of cross-linked dextran (Sephadex™) with the physical and chemical stability of highly cross-linked agarose to produce separation media with excellent selectivity and high resolution (Fig 3). In addition, the low non-specific interaction permits high recovery of biological material. The mean bead size in Superdex Peptide, Superdex 75, and Superdex 200 media is 13 µm. The small size and narrow distribution give a high resolving capacity for analytical separations.

Table 1. The main characteristics of Superdex media

	Superdex Peptide	Superdex 75	Superdex 200
Separation range (M_r)	100 to 7 000 (peptides)	3 000 to 70 000 (globular proteins)	10 000 to 600 000 (globular proteins)
Exclusion limit (M_r)	20 000	100 000 (globular proteins)	1 300 000 (globular proteins)
pH stability	1 to 14 (long- and short-term)	3 to 12 (long-term), 1 to 14 (short-term)	3 to 12 (long-term), 1 to 14 (short-term)
Temperature stability	4°C to 40°C	4°C to 40°C	4°C to 40°C
Working and storage temperature	4°C to 30°C	4°C to 30°C	4°C to 30°C
Matrix	Spherical composite of cross-linked agarose and dextran	Spherical composite of cross-linked agarose and dextran	Spherical composite of cross-linked agarose and dextran
Average particle size	13 µm	13 µm	13 µm

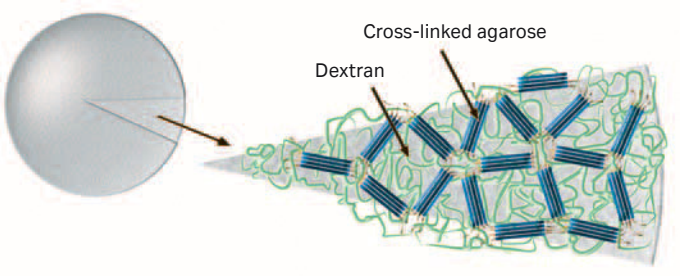


Fig 2. Schematic view through a section of a bead of Superdex. The average particle size is 13 µm. The composite dextran/cross-linked agarose matrix gives steep selectivity and high stability.

High speed gel filtration

Superdex is a composite medium with high chemical and physical stabilities. The rigid matrix withstands high pressures and allows high flow rates to be used, which will give short separation times with retained function and stability. Even shorter cycles times are made possible with Superdex 75 and 200 5/150 GL, with bed heights of 150 mm, a natural column choice for screening experiments.

Simple scale-up

Scale-up to industrial production is simple and predictable, as Superdex 30, 75, and 200 prep grade media (mean bead size 34 µm) have similar selectivities as Superdex Peptide, 75 and 200, respectively. Superdex 30, 75, and 200 prep grade media are available prepacked in HiLoad™ 16/60 and HiLoad 26/60 columns.

Figure 4 shows the scale-up of a concentrated mouse monoclonal IgG cell supernatant from a Superdex 200 high-performance column, 10 mm × 300 mm (i.d. × bed height) (Fig 4A) to HiLoad 16/60 Superdex 200 prep grade (Fig 4B). Sample volume was increased five-fold yet the separation was almost identical.

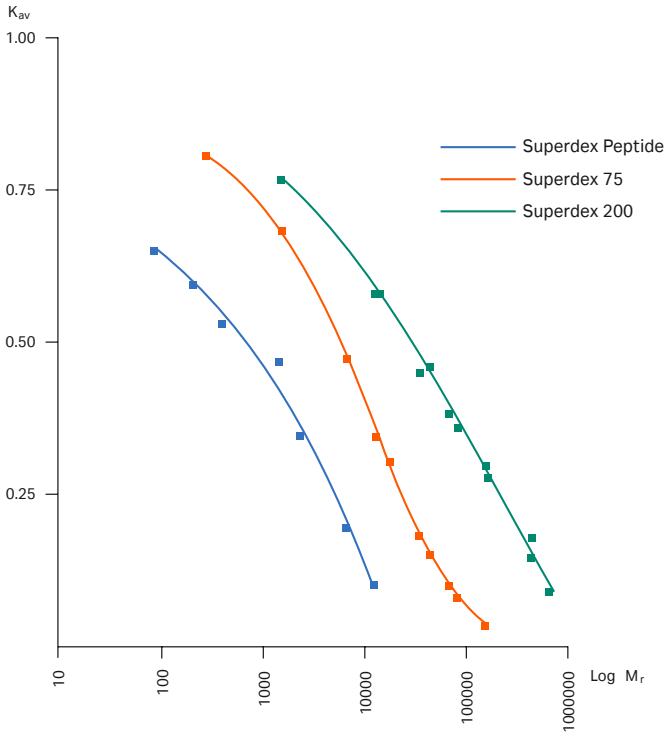


Fig 3. Selectivity curves for Superdex Peptide, 75, and 200. The steep selectivity gives high-resolution separations in the molecular weight range of 100 to 600 000.

Chemical stability

Superdex Peptide

Superdex Peptide has outstanding chemical stability and is stable in the pH range of 1 to 14. Polar organic solvents, such as 70% acetonitrile in water and methanol, and chaotropic agents such as 6 M guanidine hydrochloride or 8 M urea can also be used. The high pH stability allows separation of very hydrophobic peptides in 70% formic acid, as well as use of alkaline buffers. Exposure to repeated 48-h cycles of 1.0 M NaOH, 0.1 M HCl, 10% TFA, 70% acetonitrile, and 70% formic acid has no effect on selectivity. The recommended cleaning procedure, described in the instructions, includes the use of 0.5 M NaOH or 0.1 M HCl. Hydrophobic contaminants can be removed with 30–70% acetonitrile.

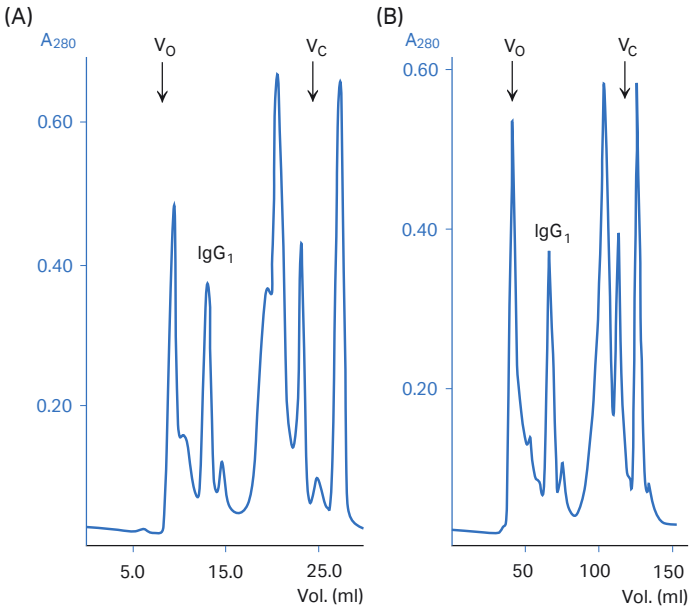


Fig 4. Simple and predictable five-fold scale up of a mouse monoclonal IgG_1 separation from a Superdex 200 high-performance column, 10 mm × 300 mm (i.d. × bed height) **(A)** to a HiLoad 16/60 Superdex 200 prep grade **(B)**. Superdex 200 prep grade medium (mean particle size 34 µm) has a similar selectivity to Superdex 200 (mean particle size 13 µm).

Superdex 75 and Superdex 200

Superdex 75 and Superdex 200 are stable in aqueous solutions over the pH range 3 to 12. Chaotropic agents (6 M guanidine hydrochloride, 8 M urea) and detergents such as SDS (up to 2%) can also be used, as well as polar organic solvents such as 30% acetonitrile. Superdex 75 and 200 withstand the conditions used for cleaning-in-place (CIP) over the pH range 1 to 14. Studies have shown that short cycles (approx. 3 h) with 0.1 M HCl or 1.0 M NaOH have no significant influence on the chromatographic behavior. However, the columns should not be stored in these solutions.

Column characteristics

Superdex media are prepacked in high-performance glass columns. Both column types, Tricorn and Precision (PC), are made of glass, which allows visual inspection of the medium bed. Tricorn columns have fittings for simple connections to ÄKTAdesign™ and other high-performance systems. Precision Columns were designed for SMART systems, but the Precision Column Holder allows use of the columns with a variety of high-performance systems. All parts of the columns are biocompatible. Table 2 summarizes the main characteristics of Tricorn and Precision columns prepacked with Superdex.

Table 2. Characteristics of Tricorn and Precision columns packed with Superdex

	Bed dimensions (i.d. × height)	Bed volume (ml)	Recommended sample volume (µl)	Theoretical plates (N/m)	Recommended flow rate[1] (ml/min)	Max. flow rate[1] (ml/min)	Max. pressure over column
Tricorn Columns							
Superdex Peptide 10/300 GL	10 × 300 mm	24	25 to 250	> 30 000	0.2 to 1.0	1.2	18 bar (261 psi, 1.8 MPa)
Superdex 75 10/300 GL	10 × 300 mm	24	25 to 250	> 30 000	0.5 to 1.0	1.5	18 bar (261 psi, 1.8 MPa)
Superdex 200 10/300 GL	10 × 300 mm	24	25 to 250	> 30 000	0.25 to 0.75	1.0	15 bar (217 psi, 1.5 MPa)
Superdex 75 5/150 GL	5 × 150 mm	3	4 to 50	> 25 000	0.15 to 0.6	0.7	18 bar (261 psi, 1.8 MPa)
Superdex 200 5/150 GL	5 × 150 mm	3	4 to 50	> 25 000	0.15 to 0.6	0.8	15 bar (217 psi, 1.5 MPa)
Precision Columns							
Superdex Peptide PC 3.2/30	3.2 × 300 mm	2.4	2 to 25	> 30 000	0.01 to 0.15	0.15	20 bar (290 psi, 2 MPa)
Superdex 75 PC 3.2/30	3.2 × 300 mm	2.4	2 to 25	> 30 000	0.01 to 0.10	0.10	24 bar (348 psi, 2.4 MPa)
Superdex 200 PC 3.2/30	3.2 × 300 mm	2.4	2 to 25	> 30 000	0.01 to 0.10	0.10	15 bar (217 psi, 1.5 MPa)

[1] H_2O at 25°C

Column sizes

Superdex columns come in two bed heights, 150 and 300 mm, thereby allowing greater flexibility to suit different analytical needs. The 150-mm prepacked Tricorn columns are designed for rapid size analysis of proteins, peptides and other biomolecules. Short cycle times together with small sample volumes and low consumption of buffers makes the column a natural choice for screening experiments, such as quick analyses of protein homogeneity. The range of 300-mm Superdex columns (see Table 2) is designed for studies where high-resolution separation of biomolecules is critical.

Excellent reproducibility and durability

Reproducible results are essential in all research. The long working life and high reproducibility of Superdex prepacked columns are the result of optimized design, the stable nature of the medium, and controlled production procedures. Figure 5 shows chromatograms of the test separation runs 1 and 210 on Superdex 75 10/300 GL.

Column:	Superdex 75 10/300 GL
Sample:	1. BSA (M_r 67 000) 8 mg/ml 2. Ovalbumin (M_r 43 000) 2.5 mg/ml, 3. Ribonuclease A (M_r 13 700) 5 mg/ml, 4. Aprotinin (M_r 6512) 2 mg/ml, 5. Vitamin B_{12} (M_r 1355) 0.1 mg/ml
Sample volume:	500 µl
Buffer:	0.05 M phosphate buffer, 0.15 M NaCl, pH 7.0
Flow rate:	0.4 ml/min, room temperature
System:	ÄKTAFPLC™

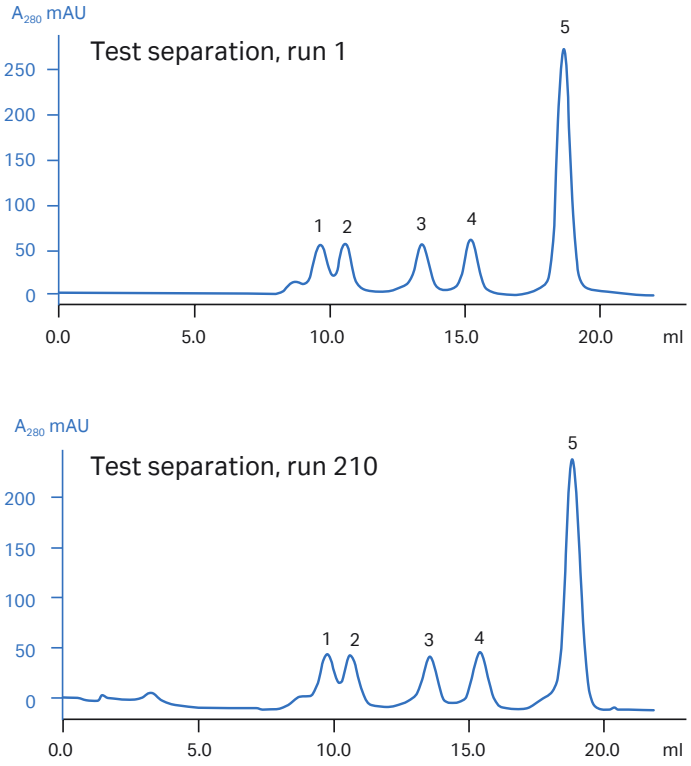


Fig 5. Comparison of the test separation runs 1 and 210 after repeated injection of a test sample on Superdex 75 10/300 GL. After 210 runs, no difference in chromatographic performance was observed.

Operation

Choice of eluents

Eluents can be chosen freely to improve recovery from separations of crude sample or to overcome solubility problems. For example, when using Superdex Peptide the sample can be made up and run in up to 70% formic acid or acetonitrile/TFA, as well as aqueous buffer solutions in the pH range 1 to 14, as demanded by the solubility of the peptides being separated. Similarly, chaotropic agents and detergents can be used to improve the solubility of membrane proteins when working with prepacked Superdex 75 or Superdex 200 columns. To avoid pH-dependent, nonionic interactions with the matrix, 0.15 M NaCl, or a buffer with equivalent ionic strength, is recommended.

Sample volumes and flow rates

The quality of a gel filtration separation is largely independent of sample concentration, but to achieve high resolution, the sample volume should be less than 5% of the total column volume. Sample volumes between 0.1% and 1.0% of the bed volume give the highest resolution. By using lower flow rates a higher resolution result can be achieved from a given column. For more information see Table 2.

Applications using Superdex Peptide

Superdex Peptide provides a powerful complement to traditional reversed-phase chromatography for separating small peptides. The excellent physical and chemical stabilities permit a wide choice of elution conditions, which extends the user's ability to separate peptides that are only soluble under special conditions. The mobile phase can also be selected to meet the requirements of detection techniques such as mass spectrometry or surface plasmon resonance.

Detecting peptide fragments

Biologically active neuropeptides in the brain are cleaved by various peptidases, causing the formation of different fragments with other biological effects. Previously, radioactive substrates or immunological techniques have been used to detect the products formed, but these methodologies lack structural specificity and are time-consuming. Gel filtration on Superdex Peptide using a SMART system, in combination with mass spectrometry, allows these studies to be carried out without such drawbacks, and all peptide fragments can be detected in a single analysis (Silberring, J., 1994, personal communication).

Separation of standard peptides

Figure 6 shows a separation of standard peptides on Superdex Peptide 10/300 GL.

Column: Superdex Peptide 10/300 GL
Sample: 1. Cytochrome c (M_r 12 384) 1 mg/ml
2. Aprotinin (M_r 6 512) 2 mg/ml
3. Vitamin B_{12} (M_r 1 355) 0.1 mg/ml
4. (Gly)3 (M_r 189) 0.1 mg/ml
5. Gly (M_r 75) 7.8 mg/ml
Flow rate: 0.5 ml/min, room temperature
Sample volume: 50 µl
Eluent: 0.05 M phosphate, 0.15 M NaCl, pH 7.0
System: ÄKTAexplorer 100

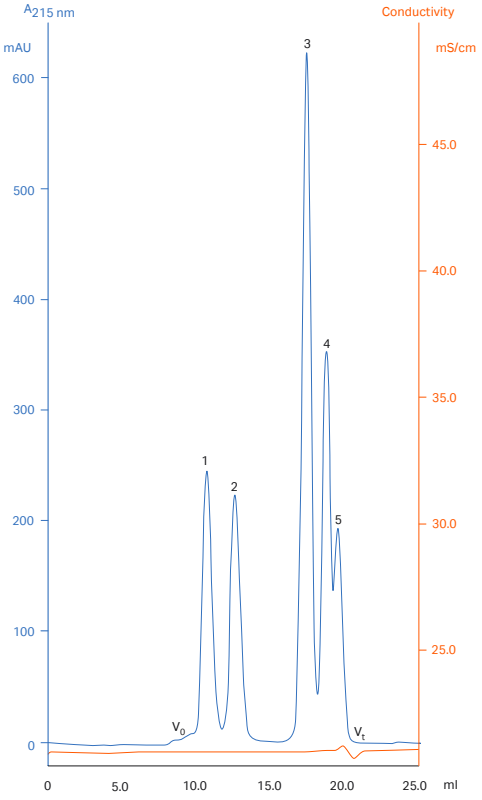


Fig 6. A separation of standard peptides on Superdex Peptide 10/300 GL.

Applications using Superdex 75 and 200 10/300 GL

Superdex 75 10/300 GL and Superdex 200 10/300 GL give high performance gel filtration of peptides and proteins within the recommended separation ranges. They can be used for any application of gel filtration, such as monitoring changes in molecular size, determining molecular weight, or as a polishing step in a purification scheme.

Separation of the monomer and dimer of a monoclonal antibody

Figure 7 shows separation of the monomer and dimer of a monoclonal antibody on Superdex 200 10/300 GL.

Dimer-monomer separation of a recombinant cystein-containing protein

A cystein-containing protein spontaneously forms dimers via a disulfide bridge. These dimers can be cleaved by adding the reducing agent dithioerythritol (DTE). The chromatogram from the Superdex 75 10/300 GL run reflects that there is a baseline separation between the monomer and dimer (Fig 8a) and that it is possible to reduce the dimers into monomers by addition of DTE (Figs 8b and 8c).

Column: Superdex 200 10/300 GL
Sample: Monoclonal antibody
Sample volume (load): 100 µl
Eluent buffer: 0.02 M Tris™ HCl, pH 7.5, 0.15 M NaCl
Flow rate: 0.25 ml/min
System: ÄKTAexplorer 100

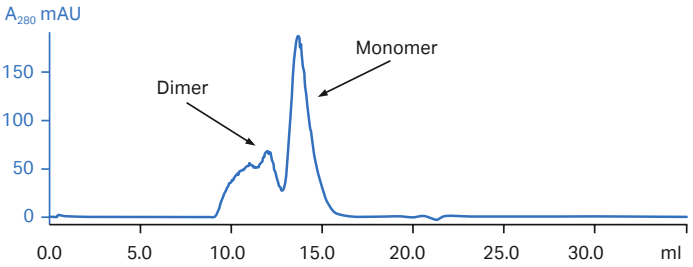


Fig 7. Separation of the monomer and dimer of a monoclonal antibody on Superdex 200 10/300 GL.

Column: Superdex 75 10/300 GL
Sample: recCys-prot
Sample volume (load): 200 µl
Eluent buffer: 0.05 M Tris HCl, 1 mM EDTA, 0.15 M NaCl, pH 8.4
Flow rate: 0.5 ml/min
System: ÄKTAexplorer 100

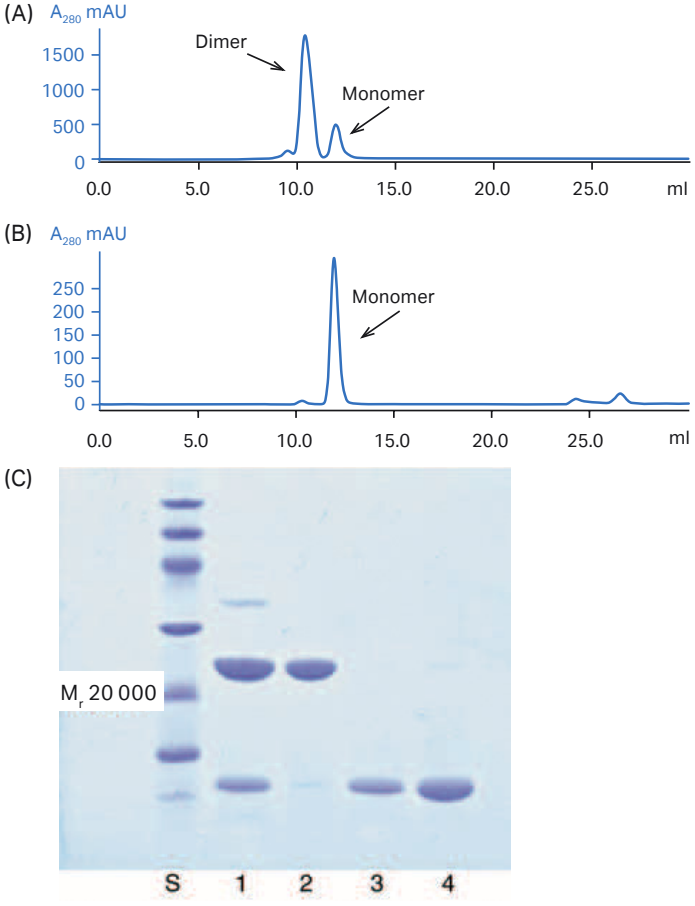


Fig 8. (A) Dimer-monomer separation of a recombinant cystein-containing-protein (recCys-prot.) on Superdex 75 10/300 GL. **(B)** purification of the dimer fraction reduced with DTE. **(C)** shows a Coomassie™ stained SDS-PAGE gel. **Lane S** is LMW-SDS Marker Kit (17-0446-01), **lane 1** is the original dimer-monomer sample; the dimer content is high, which also is reflected in the chromatogram. **Lane 2** is the dimer fraction and **lane 3** corresponds to the monomer fraction from **A**, respectively. **Lane 4** shows the monomer peak from **B**. **Lanes 1 to 4** were run under non-reducing conditions.

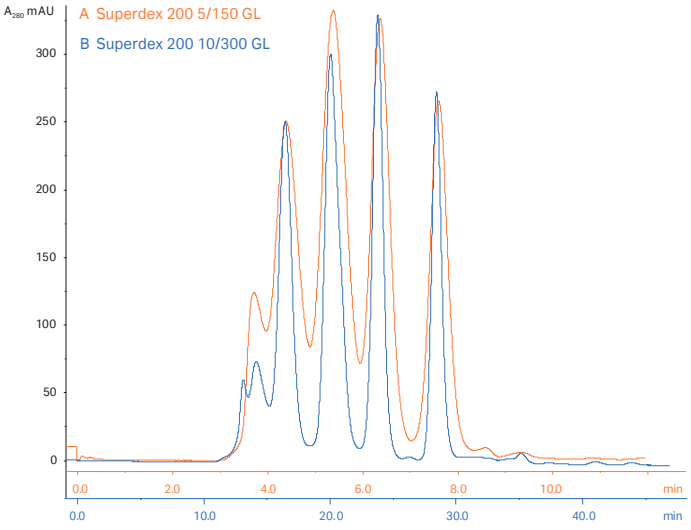
Applications using Superdex 5/150 GL

Superdex 75 and 200 5/150 GL columns have small bed volumes which is useful for a range of applications such as screening solubilization conditions for membrane purification and analyses of protein-protein interactions. Shorter Superdex columns use less buffer and sample than longer columns and are thus an excellent choice when time and sample buffer consumption are more critical than achieving high resolution.

Reduced cycle time using Superdex 5/150 GL

Figure 9 provides comparisons of analyses run on Superdex 200 5/150 GL and 10/300 GL (Fig 9 A-B), as well as Superdex 75 5/150 GL and 10/300 GL (Fig 9 C-D). Note that analyses run with Superdex 200 5/150 GL and Superdex 75 5/150 are 4 times faster than those run on Superdex 200 10/300 GL and Superdex 75 10/300, respectively. Using shorter columns that provide good resolution together with short analysis time can lead to significant cost savings. Consequently, short columns are ideal for screening. Long columns involve longer analysis time, but are preferable when high resolution analyses are required.

Column:	Superdex 200 5/150 GL (curve A below) Superdex 200 10/300 GL (curve B below)
Sample:	Ferritin (M_r 440 000), Aldolase (M_r 158 000), Ovalbumin (M_r 43 000), Ribonuclease A (M_r 13 700)
Sample volume:	12.5 µl (Superdex 200 5/150 GL) 100 µl (Superdex 200 10/300 GL)
Buffer:	PBS, pH 7.4
Flow rate:	0.3 ml/min, Superdex 200 5/150 GL 0.6 ml/min, Superdex 200 10/300 GL
Analysis time:	12 min (Superdex 200 5/150 GL) 47 min (Superdex 200 10/300 GL)
System:	ÄKTAexplorer



Analysis of protein-protein interaction

To evaluate the efficacy of Superdex 75 5/150 GL in analyzing protein-protein interactions, Trypsin and Aprotinin were run separately on Superdex 75 5/150 GL and then mixed and injected on the column (Fig 10). Only one peak eluted from the mixture of Trypsin and Aprotinin with an elution volume shifted toward the void volume, indicating protein complex formation.

Column:	Superdex 75 5/150 GL (curve C below) Superdex 75 10/300 GL (curve D below)
Sample:	Conalbumin (M_r 75 000); Carbonic Anhydrase (M_r 29 000) Ribonuclease A (M_r 13 700), Aprotinin (M_r 6 500)
Sample volume:	12.5 µl (Superdex 75 5/150 GL) 100 µl (Superdex 75 10/300 GL)
Buffer:	PBS, pH 7.4
Flow rate:	0.3 ml/min, Superdex 75 5/150 GL 0.6 ml/min, Superdex 75 10/300 GL
Analysis time:	12 min (Superdex 75 5/150 GL) 47 min (Superdex 75 10/300 GL)
System:	ETTAN LC

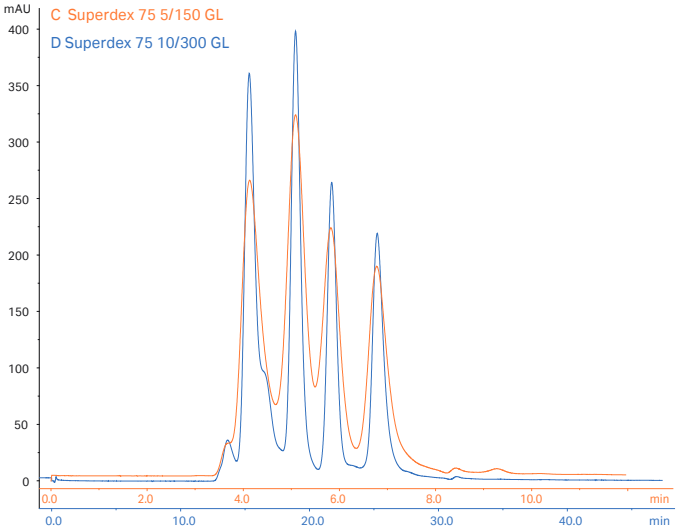


Fig 9. Comparison of analyses run on different Superdex columns. The larger 10/300 shows excellent resolution for sample analyses, while the short 5/150 column provides rapid analysis time suitable for screening experiments.

Column: Superdex 75 5/150 GL
Sample: Trypsin (M_r 23 800) 11mg/ml
Aprotinin (M_r 6 500) 3 mg/ml
Trypsin 11 mg/ml and Aprotinin 3mg/ml
Sample volume: 10 µl
Buffer: PBS, pH 7.4
Flow rate: 0.3 ml/min
System: ETTAN LC

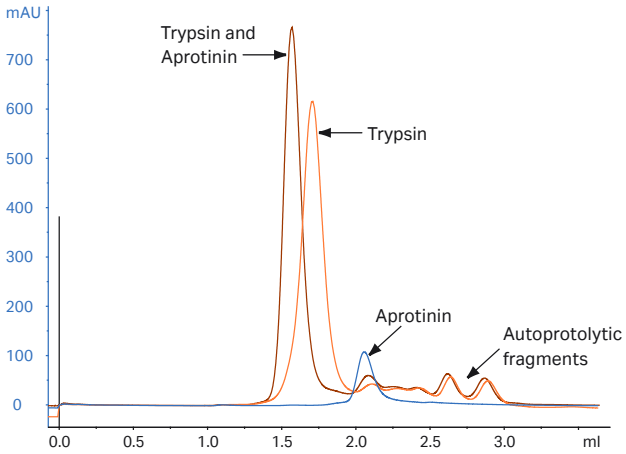


Fig 10. Monitoring of protein complex formation between Trypsin and Aprotinin.

Screening of buffer conditions for a membrane protein

Size homogeneity is a useful indicator of stability, since e.g. membrane proteins often oligomerize or aggregate rapidly when destabilized. Rapid gel filtration with Superdex 200 5/150 GL was used to screen for homogeneity under various pH and salt conditions (Fig 11).

Screening with rapid gel filtration showed a symmetrical peak when the separation was performed at pH 5.2 in 0.1 M NaCl (see Fig 11 A), indicating a homogenous protein under these conditions. At somewhat higher salt concentration (Fig 11 D) a small peak appeared close to the void volume, indicating that oligomerization or aggregation appeared to a limited extent. At both pH 7.5 and pH 9.5 significant peaks were obtained close to the void volume, indicating severe oligomerization or aggregation. The complete screening procedure was achieved in only a few hours, including the time for column equilibration. Sample consumption was 6 × 10 µl for the complete screen.

Column: Superdex 200 5/150 GL
Sample: Integral membrane protein (M_r 60 000) from *E. coli*
Sample volume: 10 µl
Eluents (including 0.1 or 0.3 M NaCl): 0.02 M sodium acetate, 0.03 % dodecyl maltoside, 0.5 mM TCEP, pH 5.2
0.02 M HEPES, 0.03 % dodecyl maltoside, 0.5 mM TCEP, pH 7.5
0.02 M CAPSO, 0.03 % dodecyl maltoside, 0.5 mM TCEP, pH 9.5
Flow rate: 0.35 ml/min
Detection: 280 nm
System: ÄKTAexplorer

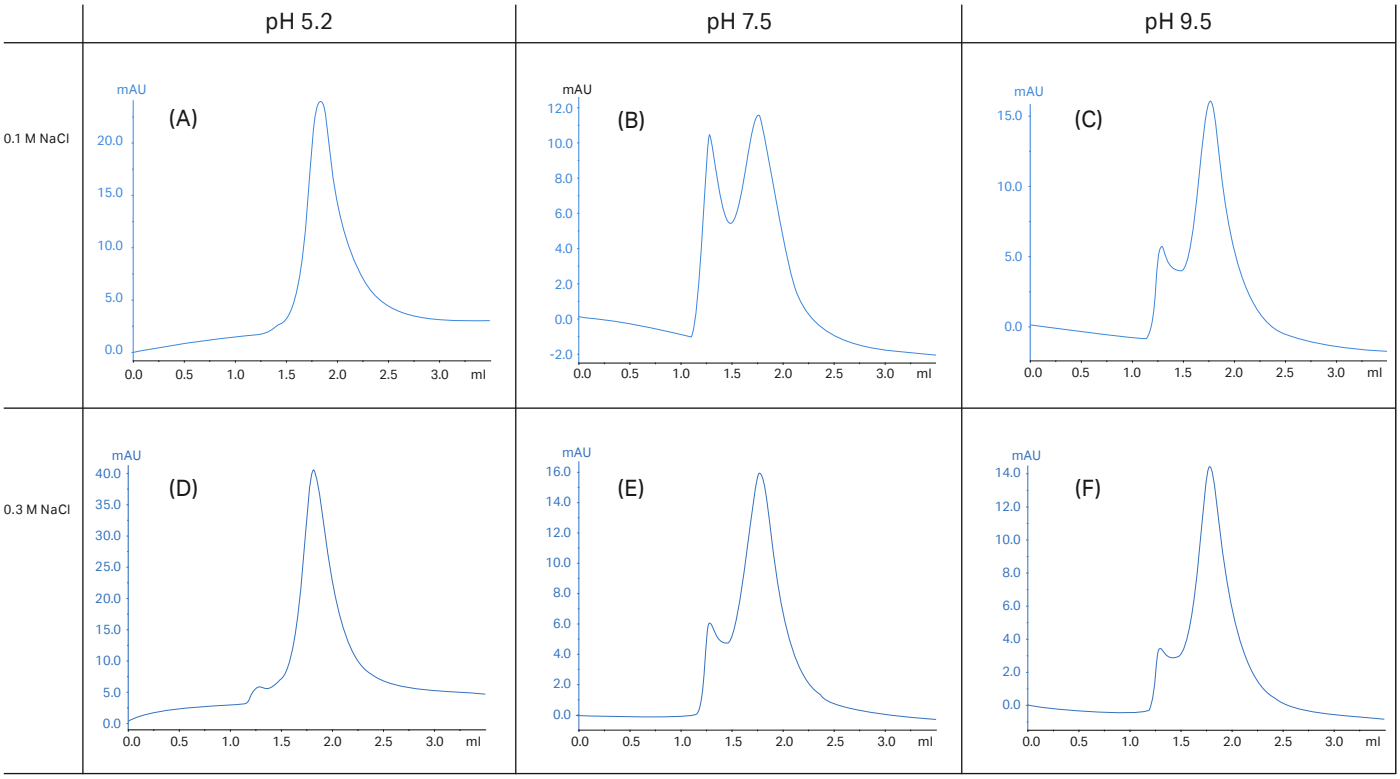


Fig 11. Screening of pH and ion strength conditions for optimal homogeneity and stability of a detergent-protein complex. Chromatogram **A–F** represent the results from the different screening conditions.

Ordering information

Product	Quantity	Code no.
Superdex Peptide 10/300 GL	1	17-5176-01
Superdex Peptide PC 3.2/30	1	17-1458-01
Superdex 75 10/300 GL	1	17-5174-01
Superdex 75 5/150 GL	1	28-9205-04
Superdex 75 PC 3.2/30	1	17-0771-01
Superdex 200 10/300 GL	1	17-5175-01
Superdex 200 5/150 GL	1	28-9065-61
Superdex 200 PC 3.2/30	1	17-1089-01

Related products

Product	Quantity	Code no.
Superdex 30 prep grade	25 ml	17-0905-10
Superdex 30 prep grade	150 ml	17-0905-01
Superdex 75 prep grade	25 ml	17-1044-10
Superdex 75 prep grade	150 ml	17-1044-01
Superdex 200 prep grade	25 ml	17-1043-10
Superdex 200 prep grade	150 ml	17-1043-01
HiLoad 16/60 Superdex 30 prep grade	1	17-1139-01
HiLoad 26/60 Superdex 30 prep grade	1	17-1140-01
HiLoad 16/60 Superdex 75 prep grade	1	17-1068-01
HiLoad 26/60 Superdex 75 prep grade	1	17-1070-01
HiLoad 16/60 Superdex 200 prep grade	1	17-1069-01
HiLoad 26/60 Superdex 200 prep grade	1	17-1071-01

Accessories

Product	Quantity	Code no.
Gel Filtration LMW Calibration Kit	1	28-4038-41
Gel Filtration HMW Calibration Kit	1	28-4038-42
Precision Column holder	1	17-1455-01

Related product literature

Literature	Code no.
Data File: HiLoad Superdex 30/75/200 prep grade	18-1100-52
Data File: Superdex 30, 75, & 200 prep grade Bioprocess Media	18-1020-92
Data File: Empty Tricorn Columns	18-1147-36
Gel Filtration Handbook	18-1022-18
Gel Filtration Selection Guide	18-1124-19
Purifying Challenging Proteins Handbook	28-9095-31

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