Superdex™ 200 Increase columns

Superdex 200 Increase prepacked columns (Fig 1) are designed for size exclusion chromatography (SEC)/high resolution gel filtration in small-scale (µg to mg), preparative purification, as well as for characterization and analysis of proteins with molecular weights (M_r) from 10 000 to 600 000. These versatile columns offer rapid separations with high resolution for a variety of applications including protein purification, aggregate analysis, studies of complex formation, and screening of samples.

Superdex 200 Increase columns offer:

- Versatile use for both preparative and analytical purposes, for a large variety of proteins
- Increased resolution compared with Superdex 200, for higher purity and improved analysis results
- Reduced runtime compared with Superdex 200, to get results faster

Size exclusion chromatography

SEC separates molecules according to their differences in size as they pass through a chromatography medium packed in a column. Unlike ion exchange or affinity chromatography, molecules do not bind to the chromatography medium and the buffer composition has no direct effect on resolution (the degree of separation between peaks). Consequently, a significant advantage of SEC is that conditions can be varied to suit the sample type or the requirements for further purification, analysis, or storage, without altering the separation.

SEC is an excellent technique for discriminating between monomer, oligomer, and aggregated forms of a target protein.

Fig 1. Superdex 200 Increase 3.2/300, Superdex 200 Increase 5/150 GL, and Superdex 200 Increase 10/300 GL columns.

Medium characteristics

The chromatography medium in Superdex 200 Increase columns is based on a high-flow agarose base matrix with good pressure/flow properties and a small bead size (average bead size 8.6 µm). In addition, the low nonspecific interaction permits high recovery of biological materials. The small bead size allows for high resolving analytical separations. The characteristics of the Superdex 200 Increase medium are shown in Table 1.

Table 1. Characteristics of Superdex 200 Increase medium

Fractionation range	M_r 10 000 to 600 000 (globular proteins) M_p* 1000 to 100 000 (dextrans)
Exclusion limit	M_r 1 300 000 (globular proteins)
pH stability	3 to 12 (long-term) 1 to 14 (short-term)
Operating temperature	4°C to 40°C
Storage temperature	4°C to 30°C
Matrix	Composite of cross-linked agarose and dextran
Average bead size	8.6 µm

* Peak molecular weight

In a separation of a sample mixture of four model proteins on Superdex 200 Increase 10/300 GL, the selectivity curve shows a linear and very steep slope implying a high resolution (Fig 2).

Column: Superdex 200 Increase 10/300 GL
Sample: 1. Ribonuclease A (M_r 13 700), 2 mg/ml
2. Ovalbumin (M_r 44 000), 4 mg/ml
3. Aldolase (M_r 158 000), 3 mg/ml
4. Ferritin (M_r 440 000), 0.3 mg/ml
Sample volume: 50 µl
Flow rate: 1.0 ml/min
Buffer: PBS (0.01 M phosphate buffer, 0.14 M NaCl, pH 7.4)
System: ÄKTAmicro™

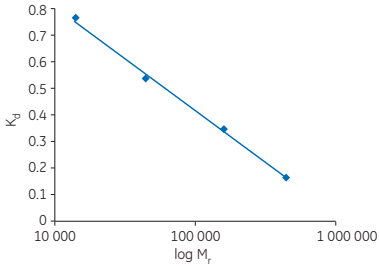

Fig 2. Selectivity curve for Superdex 200 Increase medium. The steep slope implies high-resolution separation of proteins in the M_r range 10 000 to 600 000.

Chemical stability of Superdex 200 Increase

Superdex 200 Increase medium is stable in aqueous solutions over the pH range 3 to 12. Solutions containing chaotropic agents (6 M guanidine hydrochloride, 8 M urea) and detergents such as SDS (up to 2%) can be used together with the medium without affecting its performance. Superdex 200 Increase medium also withstands the conditions used for CIP in the pH range 1 to 14. Studies have shown that short CIP cycles (approx. 3 h) with 0.1 M HCl, 1.0 M NaOH, or organic solvents such as 30% acetonitrile have no significant influence on the chromatographic performance, however, the medium should not be stored in these solutions.

Column characteristics

Superdex 200 Increase medium is available prepacked in high-performance glass columns of three different sizes (Table 2). Glass columns are used to allow visual inspection of the bed. The columns have fittings for simple connection to ÄKTA™ chromatography systems or equivalent high-performance systems. All column parts are biocompatible. Superdex 200 Increase columns come in two bed heights, 150 and 300 mm, and the longer columns come in two different inner diameters, thereby allowing for greater flexibility to suit different application needs.

Column selection

Each column has its own application purpose. Superdex 200 Increase 10/300 GL is suitable for both small-scale (mg), preparative, and high-resolution analysis applications. Superdex 200 Increase 5/150 GL is designed for rapid purity checking and quick screening of different conditions. Results can be achieved within 7 min with good resolution. Superdex 200 Increase 3.2/300 is the choice for both preparative (µg scale) purposes and high-resolution analyses, especially when limited amount of sample is available. Even a small amount of sample will give a good detection signal. An overview of what column to choose for different applications is shown in Table 3.

Table 2. Characteristics of columns prepacked with Superdex 200 Increase

Column	Bed dimensions diam. x height (mm)	Approximate bed volume (ml)	Recommended sample volume (µl)	Theoretical plates (N/m)	Recommended flow rate (ml/min)	Max. flow rate H_2O at 25° (ml/min)	Typical pressure over column* (MPa)
Superdex 200 Increase 10/300 GL	10 x 300	24	25 to 500	48 000	0.75	1.8	3.0
Superdex 200 Increase 5/150 GL	5 x 150	3	4 to 50	42 000	0.45	0.75	3.0
Superdex 200 Increase 3.2/300	3.2 x 300	2.4	4 to 50	48 000	0.075	0.15	3.0

* Pressure at maximum flow rate in water at 25°C.

Table 3. Column choice for different applications

Type of application/column	10/300 GL	5/150 GL	3.2/300
Small-scale preparative runs (mg)	×		
Micro-scale preparative runs (µg) When sample amount is limited and small consumption of buffer is important			×
High-resolution analysis (25 to 500 µl)	×		
High-resolution analysis (4 to 25 µl) When sample amount is limited and small consumption of buffer is important			×
Purity check		×	
Rapid screening When time is limited and small buffer and sample consumptions are important		×	

High dynamic range of sample load for small-scale, preparative applications

The Superdex 200 Increase 10/300 GL column has a high sample load capacity with retained high resolution. A 500 µl sample mix, containing seven model proteins, was applied to the column. As shown in Figure 3, the resolution was very good even with a high load of total protein (9.2 mg).

Column:	Superdex 200 Increase 10/300 GL
Sample:	1. Thyroglobulin (M_r 669 000), 3 mg/ml 2. Ferritin (M_r 440 000), 0.3 mg/ml 3. Aldolase (M_r 158 000), 3 mg/ml 4. Conalbumin (M_r 75 000), 3 mg/ml 5. Ovalbumin (M_r 44 000), 3 mg/ml 6. Carbonic anhydrase (M_r 29 000), 3 mg/ml 7. Ribonuclease A (M_r 13 700), 3 mg/ml
Sample volume:	500 µl
Flow rate:	0.5 ml/min
Buffer:	PBS (0.01 M phosphate buffer, 0.14 M NaCl, pH 7.4)
System:	ÄKTA explorer

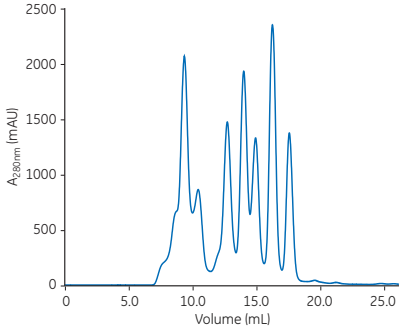

Fig 3. High-sample load (500 µl) separation of a mixture containing seven proteins on Superdex 200 Increase 10/300 GL.

Small sample volumes and low buffer consumption

Superdex 200 Increase 3.2/300 gives high resolution and good sensitivity for small sample volumes. A 20 µl sample mixture, containing seven model proteins, was applied both to Superdex 200 Increase 3.2/300 and 10/300 GL columns. As shown in Figures 4 A and B, the detection signal from the run with Superdex 200 Increase 3.2/300 was considerably higher compared to that with Superdex 200 Increase 10/300 GL.

Column:	(A) Superdex 200 Increase 3.2/300 (B) Superdex 200 Increase 10/300 GL
Sample:	1. Thyroglobulin (M_r 669 000), 3 mg/ml 2. Ferritin (M_r 440 000), 0.3 mg/ml 3. Aldolase (M_r 158 000), 3 mg/ml 4. Conalbumin (M_r 75 000), 3 mg/ml 5. Ovalbumin (M_r 44 000), 3 mg/ml 6. Carbonic anhydrase (M_r 29 000), 3 mg/ml 7. Ribonuclease A (M_r 13 700), 3 mg/ml
Sample volume:	20 µl
Flow rate:	(A) 0.05 ml/min; (B) 0.5 ml/min
Buffer:	PBS (0.01 M phosphate buffer, 0.14 M NaCl, pH 7.4)
System:	ÄKTAmicro

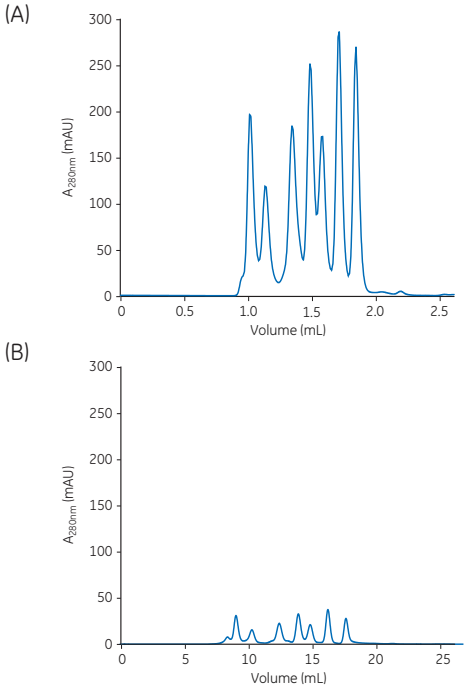

Fig 4. A small volume (20 µl with a total of 370 µg protein) of a sample mixture containing seven proteins was separated on (A) Superdex 200 Increase 3.2/300 column and (B) Superdex 200 Increase 10/300 GL column. Both graphs show the same scale for UV detection.

Reduced cycle time using Superdex 200 Increase 5/150 GL

Superdex 200 Increase 5/150 GL is designed for rapid size analysis and purity check. The effect of flow rate on cycle time and resolution of monoclonal antibody (MAb) monomers and dimers was studied and the results are shown in Figure 5. The MAb was analyzed at three different flow rates, 0.6, 0.45, and 0.15 ml/min, resulting in cycle times of 6, 8, and 23 min, respectively. The recommended flow rate of 0.45 ml/min gave a good separation of monomers and dimers (Fig 5 B and E).

Column:	Superdex 200 Increase 5/150 GL
Sample:	3.4 mg/ml MAb
Sample volume:	50 µl
Flow rate:	(A) 0.6 ml/min (B) 0. 45 ml/min (C) 0.15 ml/min
Buffer:	PBS (0.01 M phosphate buffer, 0.14 M NaCl, pH 7.4)
System:	ÄKTAmicro

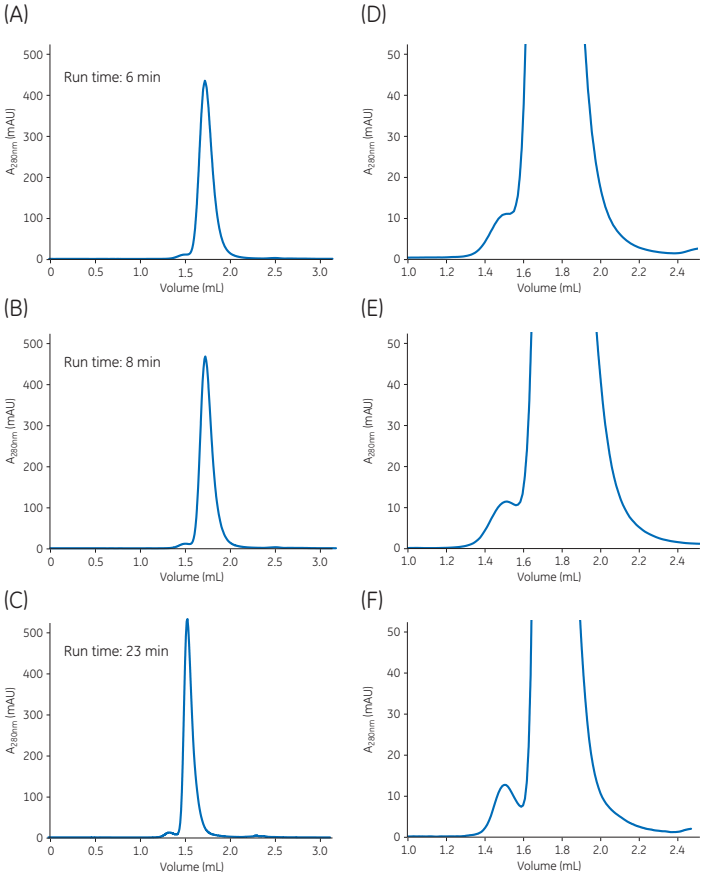

Fig 5. Effect of flow rate on cycle time and resolution of MAb monomers and dimers using Superdex 200 Increase 5/150 GL. (A) Run at a flow rate of 0.6 ml/min, resulting in a 6 min separation time. (B) Run at recommended flow rate 0.45 ml/min, resulting in an 8 min separation time. (C) Run at 0.15 ml/min, resulting in a 23 min separation time. D, E, and F are amplifications of A, B, and C chromatograms. For detection, absorbance was measured at 280 nm.

Excellent reproducibility and durability

Reproducible results are essential in all research. The long working life and high reproducibility of Superdex 200 Increase prepacked columns are the result of optimized design, stable nature of the medium, and controlled production procedures. A test was performed to verify the chemical stability and robustness of the medium and prepacked column. Initially the column was tested with standard proteins (Fig 6A) followed by a series of runs with IgG sample with subsequent CIP procedure after each run. After 150 runs a new test with standard proteins was performed (Fig 6B). The results show very similar chromatograms for both the initial run and run 150.

Determination of theoretical plates (N/m) was performed to evaluate the column efficiency after each run. Results show that the column efficiency is stable over time (Fig 7). This shows that Superdex 200 Increase medium is chemically stable and withstands high pH variations (pH 1 to 14 short-term) without loss in physical or functional performance.

Column:	Superdex 200 Increase 10/300 GL
Sample:	1. Dextran blue (M_r 2 000 000), 2 mg/ml 2. Thyroglobulin (M_r 669 000), 2 mg/ml 3. Aldolase (M_r 158 000), 2 mg/ml 4. Ovalbumin (M_r 44 000), 2 mg/ml 5. Ribonuclease (M_r 13 700), 0.6 mg/ml
Sample volume:	100 µl
Flow rate:	0.5 ml/min
Buffer:	PBS (0.01 M phosphate buffer, 0.14 M NaCl, pH 7.4)
System:	ÄKTA explorer
CIP:	1 M NaOH

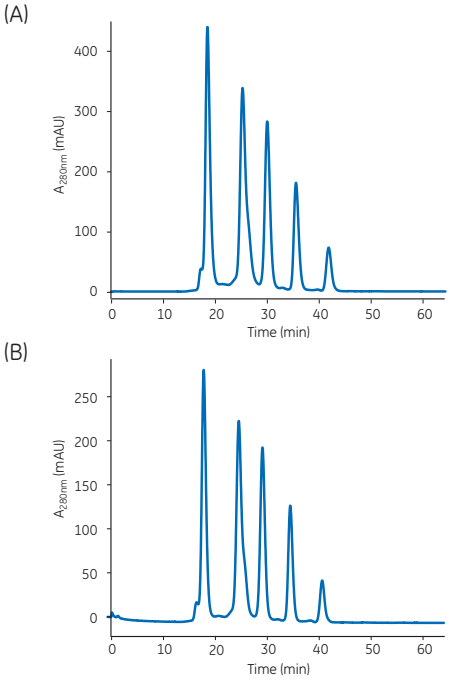

Fig 6. Comparison of a test separation of standard proteins. (A) Initial run and (B) run 150 after repeated injection of a IgG test sample and CIP cycles on Superdex 200 Increase 10/300 GL.

Column:	Superdex 200 Increase 10/300 GL
Sample:	2% acetone in 20% ethanol
Sample volume:	100 µl
Flow rate:	0.6 ml/min
Eluent:	20% ethanol
System:	ÄKTA explorer 10

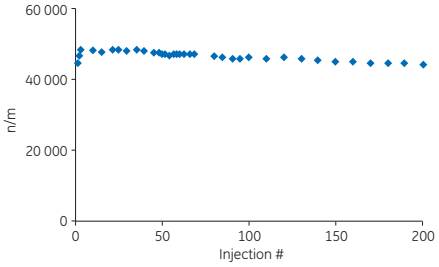

Fig 7. The plate number per meter (N/m) plotted against the injection number.

Operation

Choice of eluents

An eluent that ensures the sample is fully soluble and, if possible, that will simplify downstream applications, should be selected. For example chaotropic agents and detergents can be used to improve the solubility of membrane proteins. To further minimize the very low ionic interactions with the matrix, 0.15 M NaCl or a buffer with equivalent ionic strength is recommended.

Sample volumes and flow rates

When working with protein concentrations below 10 mg/ml, the gel filtration separation becomes less dependent on sample concentration. To achieve high resolution, the sample volume should be less than 2% of the total column volume. Sample volumes between 0.1% and 1.0% of the bed volume give the highest resolution. By decreasing flow rate, an increasing resolution can be achieved from a given column. For more information see Table 2.

Applications

Analysis of MAb homogeneity

A critical step in MAb production and characterization is the analysis of aggregates and determination of purity of the monomeric fraction. Figures 8 A and B illustrates the separation of a monoclonal mouse IgG antibody from its aggregates using Superdex 200 Increase 10/300 GL. As shown, the aggregates and dimers were very well separated from the monomeric peak.

Column:	Superdex 200 Increase 10/300 GL
Sample:	Mouse IgG_1, 1.0 mg/ml
Sample volume:	15 µl
Flow rate:	0.6 ml/min
Buffer:	0.1 M phosphate, 0.2 M NaCl, pH 6.8
System:	HPLC system

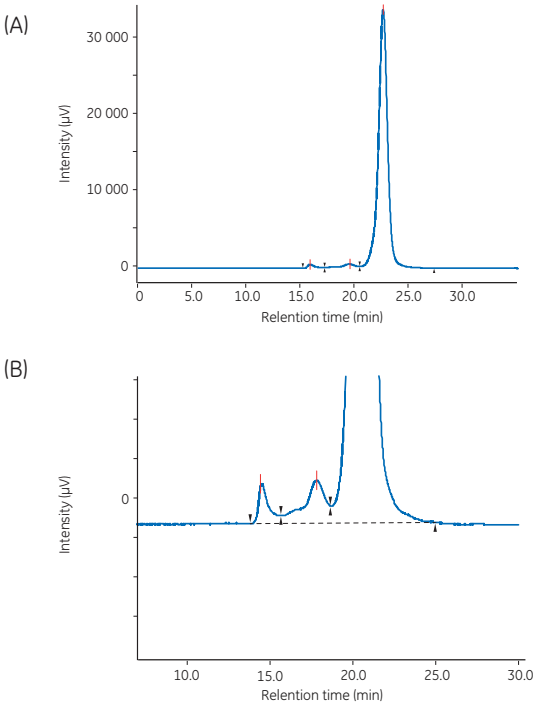

Fig 8. Analysis of monoclonal mouse IgG_1 antibody aggregates using Superdex 200 Increase 10/300 GL. (A) Chromatogram without magnification. (B) Magnified chromatogram showing details of aggregates, dimer, and monomer peaks.

Analysis of antibody aggregates, monomers, and fragments

MAb fragments are getting increasingly interesting for research use because of their small size and lower immunogenicity relative to intact antibodies. The Superdex 200 Increase 10/300 GL column was used for analysis in the development of an improved workflow for producing Fab fragments. The fragments were generated by papain cleavage of a purified MAb followed by purification on MabSelect SuRe™ and Capto™ L media. Fractions from different steps were analyzed using gel filtration. Figure 9 shows an overlay from analyses of purified intact MAb and purified Fab fragment.

Column:	Superdex 200 Increase 10/300 GL
Sample:	1. Intact MAb (M_r 150 000) 2. Fab fragment (M_r 45 000)
Sample volume:	50 µl
Flow rate:	0.5 ml/min
Buffer:	PBS (0.01 M phosphate buffer, 0.14 M NaCl, pH 7.4)
System:	ÄKTA pure

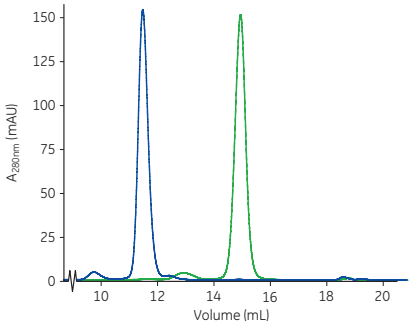

Fig 9. Overlay of two chromatograms for analysis of a papain-digested IgG antibody using Superdex 200 Increase 10/300 GL. Blue chromatogram corresponds to intact MAb and green chromatogram to Fab fragment.

Rapid detergent screening for membrane proteins

For evaluation of detergents for further crystallization of a membrane protein, a rapid screening of maltoside detergents of variable acyl chain length (DDM, UDM, DM, NM) was performed on Superdex 200 Increase 5/150 GL. Purified recombinant membrane protein CE07 was run on Superdex 200 Increase 5/150 GL, equilibrated in respective detergent. The results are shown in Figure 10. As seen from the chromatograms, the DDM run gave the most homogenous peak and is therefore the best choice for further crystallization experiments.

Column:	Superdex 200 Increase 5/150 GL
Sample:	Recombinant integral membrane protein CE07
Sample volume:	25 µl
Flow rate:	0.5 ml/min
Buffer:	20 mM Hepes, 150 mM NaCl, 5% glycerol, 0.5 mM TCEP + maltoside detergent, pH 7.5
Detergent:	0.03% DDM, 0.2% UDM, 0.01% DM, or 0.3% NM
System:	ÄKTAmicro

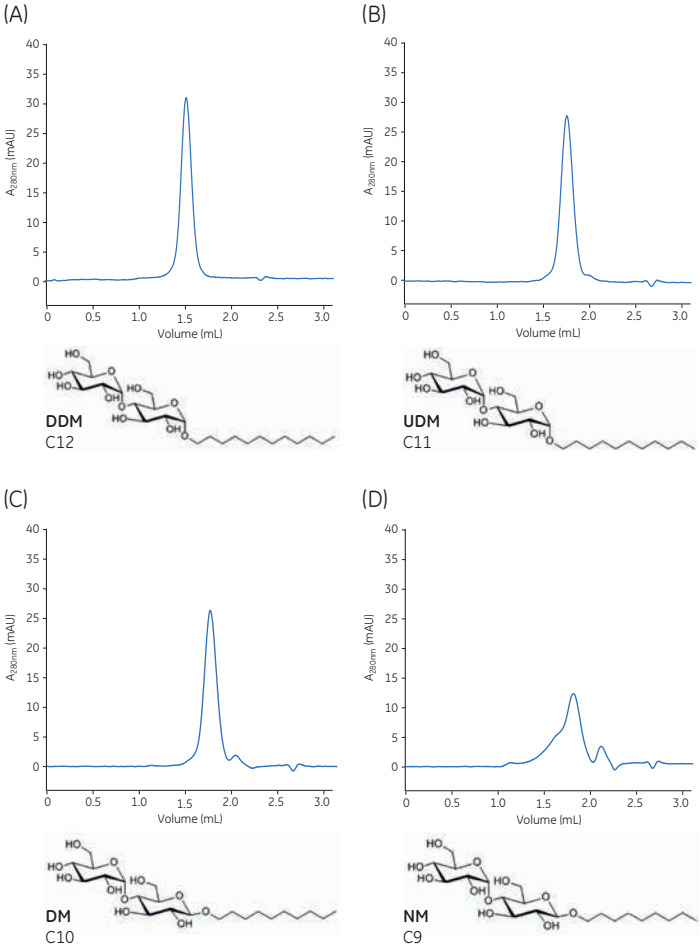

Fig 10. Rapid screening of detergents using Superdex 200 Increase 5/150 GL. Purified recombinant membrane protein CE07 was run in a column equilibrated with (A) DDM, (B) UDM, (C) DM, and (D) NM.

Analysis of protein-protein interaction

Superdex 200 Increase 10/300 GL was used to monitor complex formations. Trypsin and soybean trypsin inhibitor were run separately as well as in a mixture on the column. Overlapping chromatograms of three runs are shown in Figure 11. The major peak eluting early in the mixture run corresponds to complex formation between trypsin and soybean trypsin inhibitor.

Column:	Superdex 200 Increase 10/300 GL
Sample:	A. Soybean trypsin inhibitor (M_r 20 100) 1 mg/ml B. Trypsin (M_r 23 300) 1 mg/ml C. Mixture of A and B
Sample volume:	50 µl
Flow rate:	0.75 ml/min
Buffer:	PBS (0.01 M phosphate buffer, 0.14 M NaCl, pH 7.4)
System:	ÄKTAmicro

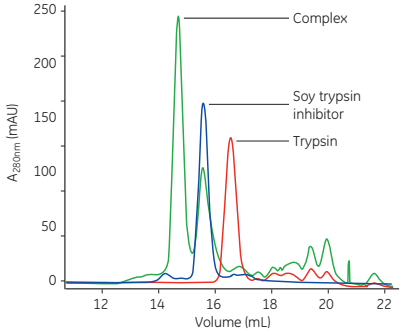

Fig 11. Monitoring protein complex formation by analysis on Superdex 200 Increase 10/300 GL. Green chromatogram shows the complex formed between trypsin and soybean trypsin inhibitor. Blue chromatogram shows the soybean trypsin inhibitor peak. Red chromatogram shows the trypsin peak. The complex migrates fast, elutes early, and is shown as a large, green peak.

Ordering information

Product	Quantity	Code number
Superdex 200 Increase 10/300 GL	1	28-9909-44
Superdex 200 Increase 5/150 GL	1	28-9909-45
Superdex 200 Increase 3.2/300	1	28-9909-46

Related products

Gel Filtration LMW Calibration Kit	1	28-4038-41
Gel Filtration HMW Calibration Kit	1	28-4038-42

Accessories

Tricorn™ 10 Filter Kit	1	18-1153-11
Tricorn 5 Filter Kit	1	18-1153-02
Filter Tool	1	18-1153-20
Fingertight connector, 1/16" male	10	18-1112-55
Tricorn storage/shipping device	1	18-1176-43

Related literature

Handbook: Size Exclusion Chromatography, Principles and Methods	18-1022-18
Selection Guide: Size Exclusion Chromatography Filtration Columns and Media	18-1124-19
Selection Guide: Prepacked chromatography columns for ÄKTA systems	28-9317-78

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