# **Superdex** 200 Increase columns

SIZE EXCLUSION CHROMATOGRAPHY

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 resin packed in a column. Unlike ion exchange or affinity chromatography, molecules do not bind to the chromatography resin 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.

## Resin characteristics

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



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

**Table 1.** Characteristics of Superdex 200 Increase resin

| | |
|---|---|
| Matrix | Composite of cross-linked agarose and dextran |
| Fractionation range | $M_r$ 10 000 to 600 000 (globular proteins)<br>$M_p$* 1000 to 100 000 (dextrans) |
| Exclusion limit | $M_r$ 1 300 000 (globular proteins) |
| Particle size, $d_{50v}$† | 8.6 µm |
| pH stability | Operational‡ 3 to 12<br>Cleaning-in-place (CIP)¶ 1 to 14 |
| Operating temperature | 4°C to 40°C |
| Storage temperature | 4°C to 30°C |

* Peak molecular weight.

† Median particle size of the cumulative volume distribution.

‡ pH range where resin can be operated without significant change in function.

¶ pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

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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<br>2. Ovalbumin ($M_r$ 44 000), 4 mg/mL<br>3. Aldolase ($M_r$ 158 000), 3 mg/mL<br>4. Ferritin ($M_r$ 440 000), 0.3 mg/mL |
| *Sample volume:* | 50 µL |
| *Buffer:* | PBS (0.01 M phosphate buffer, 0.14 M NaCl, pH7. 4) |
| *Flow rate:* | 1.0 mL/min |
| *System:* | ÄKTAmicro |

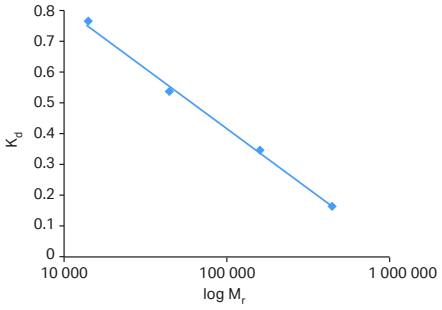



**Fig 2.** Selectivity curve for Superdex 200 Increase resin. The steep slope implies high-resolution separation of proteins in the $M_r$ range 10 000 to 600 000.

## Chemical stability of Superdex 200 Increase

Superdex 200 Increase resin 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 resin without affecting its performance. Superdex 200 Increase resin 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 resin should not be stored in these solutions.

## Column characteristics

Superdex 200 Increase resin 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 | 2.0 |

* 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)** | × | | |
| **Microscale preparative runs (µg)**<br>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)**<br>When sample amount is limited and small consumption of buffer is important | | | × |
| **Purity check** | | × | |
| **Rapid screening**<br>When time is limited and small buffer and sample consumptions are important | | × | |

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## 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<br>2. Ferritin ($M_r$ 440 000), 0.3 mg/mL<br>3. Aldolase ($M_r$ 158 000), 3 mg/mL<br>4. Conalbumin ($M_r$ 75 000), 3 mg/mL<br>5. Ovalbumin ($M_r$ 44 000), 3 mg/mL<br>6. Carbonic anhydrase ($M_r$ 29 000), 3 mg/mL<br>7. Ribonuclease A ($M_r$ 13 700), 3 mg/mL |
| *Sample volume:* | 500 µL |
| *Buffer:* | PBS (0.01 M phosphate buffer, 0.14 M NaCl, pH 7.4) |
| *Flow rate:* | 0.5 mL/min |
| *System:* | ÄKTA explorer |

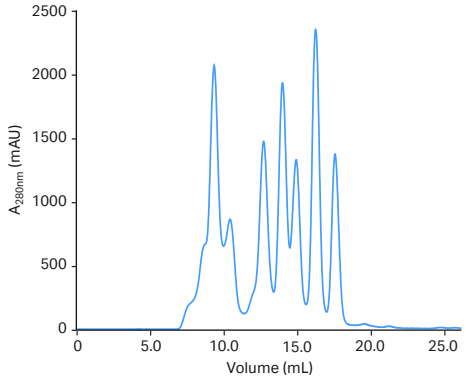



**Fig 3.** High-sample load (500 µL) separation of a mixture containing seven proteins on Superdex 200 Increase 10/300 GL.

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

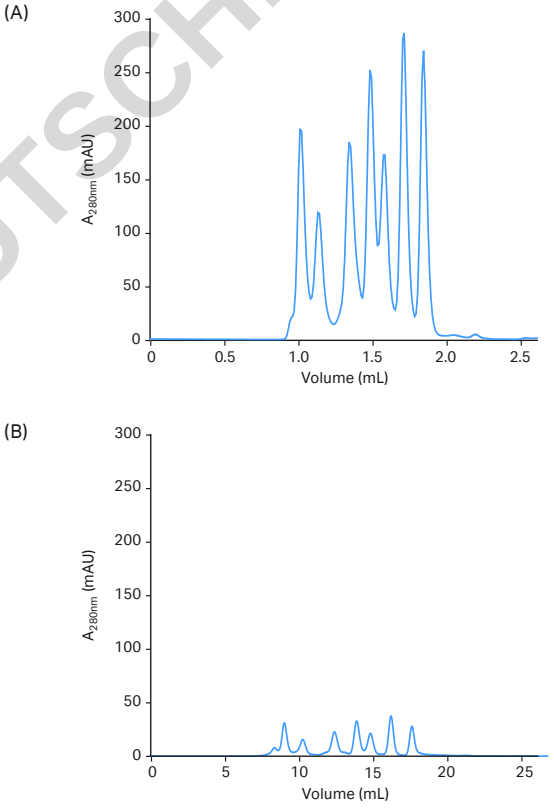



**Fig 4.** A small volume (20 µL with a total of 370 µg protein) of a sample mixture containing seven proteins was separated on (A) Superdex 200 Increase 3.2/300 column and (B) Superdex 200 Increase 10/300 GL column. Both graphs show the same scale for UV detection.

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## 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 |
| *Buffer:* | PBS (0.01 M phosphate buffer, 0.14 M NaCl, pH 7.4) |
| *Flow rate:* | (A) 0.6 mL/min<br>(B) 0. 45 mL/min<br>(C) 0.15 mL/min |
| *System:* | ÄKTAmicro |

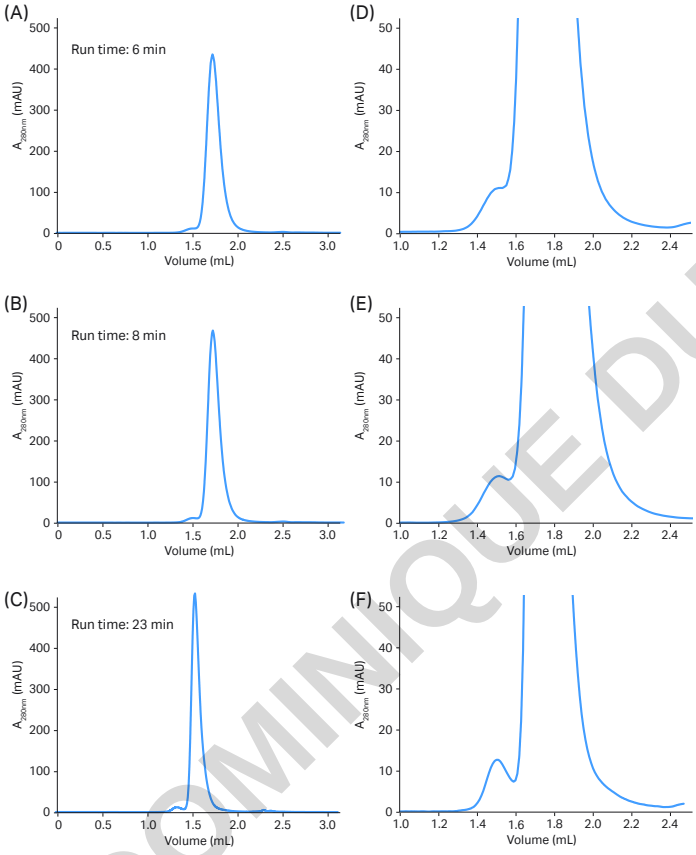



**Fig 5.** Effect of flow rate on cycle time and resolution of mAb monomers and dimers using Superdex 200 Increase 5/150 GL. (A) Run at a flow rate of 0.6 mL/min, resulting in a 6 min separation time. (B) Run at recommended flow rate 0.45 mL/min, resulting in an 8 min separation time. (C) Run at 0.15 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 resin, and controlled production procedures. A test was performed to verify the chemical stability and robustness of the resin 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 resin is chemically stable and withstands high pH variations (pH 1 to 14 cleaning in place) 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<br>2. Thyroglobulin ($M_r$ 669 000), 2 mg/mL<br>3. Aldolase ($M_r$ 158 000), 2 mg/mL<br>4. Ovalbumin ($M_r$ 44 000), 2 mg/mL<br>5. Ribonuclease ($M_r$ 13 700), 0.6 mg/mL |
| *Sample volume:* | 100 µL |
| *Buffer:* | PBS (0.01 M phosphate buffer, 0.14 M NaCl, pH 7.4) |
| *Flow rate:* | 0.5 mL/min |
| *System:* | ÄKTA explorer |
| *CIP:* | 1 M NaOH |

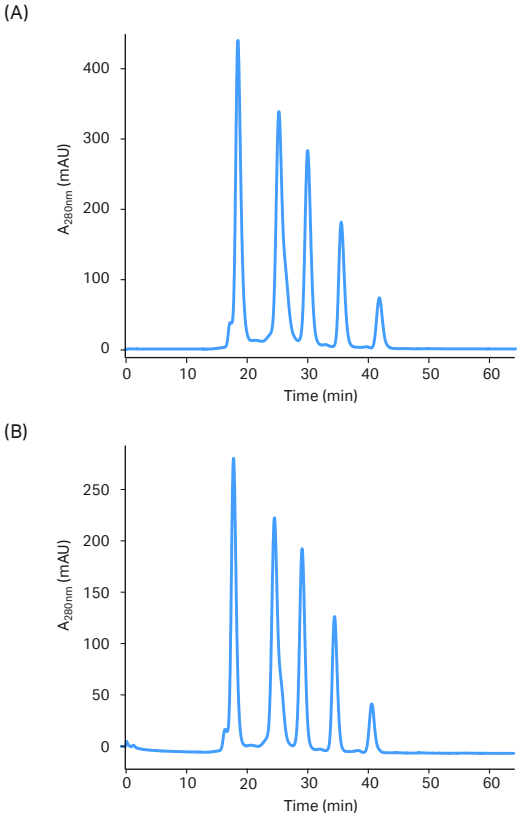



**Fig 6.** Comparison of a test separation of standard proteins. (A) Initial run and (B) run 150 after repeated injection of a IgG test sample and CIP cycles on Superdex 200 Increase 10/300 GL.

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| | |
|---|---|
| *Column:* | Superdex 200 Increase 10/300 GL |
| *Sample:* | 2% acetone in 20% ethanol |
| *Sample volume:* | 100 µL |
| *Eluent:* | 20% ethanol |
| *Flow rate:* | 0.6 mL/min |
| *System:* | ÄKTA explorer 10 |

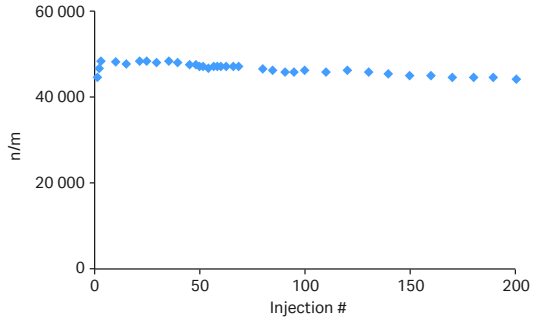



**Fig 7.** The plate number per meter (N/m) plotted against the injection number.

# 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 size exclusion chromatography 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 |
| *Buffer:* | 0.1 M phosphate, 0.2 M NaCl, pH 6.8 |
| *Flow rate:* | 0.6 mL/min |
| *System:* | HPLC system |

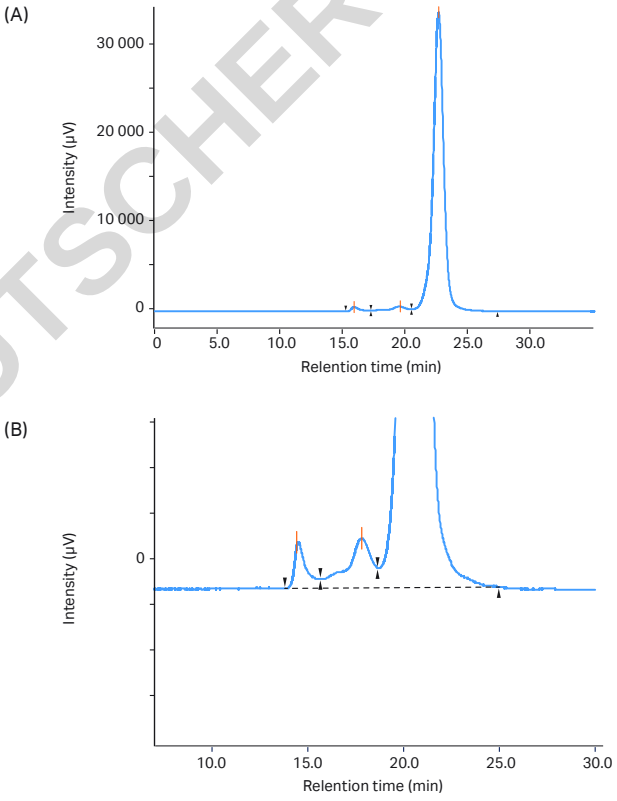



**Fig 8.** Analysis of monoclonal mouse $IgG_1$ antibody aggregates using Superdex 200 Increase 10/300 GL. (A) Chromatogram without magnification. (B) Magnified chromatogram showing details of aggregates, dimer, and monomer peaks.

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# Analysis of antibody aggregates, monomers, and fragments

mAb fragments are 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 resins. Fractions from different steps were analyzed using size exclusion chromatography. 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)<br>2. Fab fragment ($M_r$ 45 000) |
| *Sample volume:* | 50 µL |
| *Buffer:* | PBS (0.01 M phosphate buffer, 0.14 M NaCl, pH 7.4) |
| *Flow rate:* | 0.5 mL/min |
| *System:* | ÄKTA pure |

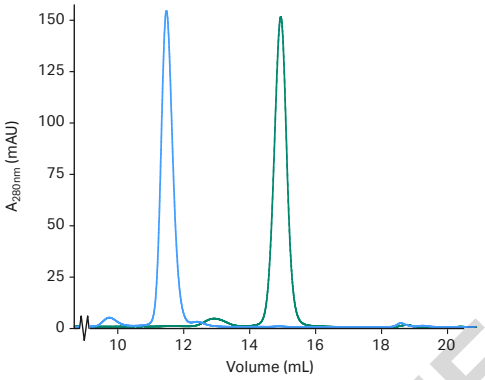



**Fig 9.** Overlay of two chromatograms for analysis of a papain-digested IgG antibody using Superdex 200 Increase 10/300 GL. Blue chromatogram corresponds to intact mAb and green chromatogram to Fab fragment.

# Rapid detergent screening for membrane proteins

For evaluation of detergents for further crystallization of amembrane 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 |
| *Buffer:* | 20 mM HEPES, 150 mM NaCl, 5% glycerol,<br>0.5 mM TCEP + maltoside detergent, pH 7.5 |
| *Flow rate:* | 0.5 mL/min |
| *Detergent:* | 0.03% DDM, 0.2% UDM, 0.01% DM, or 0.3% NM |
| *System:* | ÄKTAmicro |

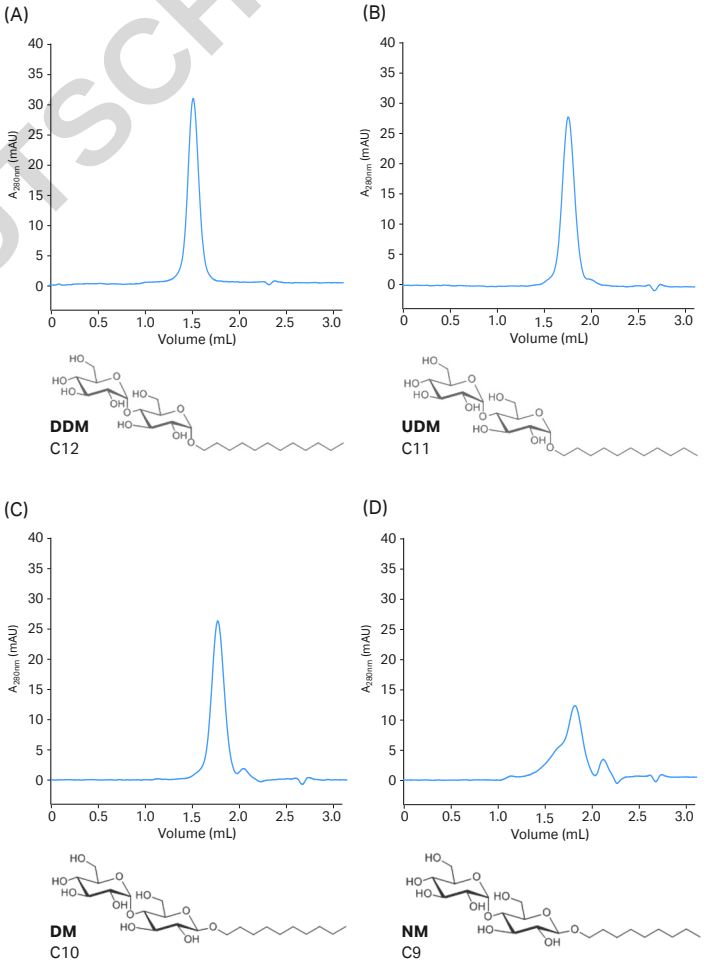



**Fig 10.** Rapid screening of detergents using Superdex 200 Increase 5/150 GL. Purified recombinant membrane protein CE07 was run in a column equilibrated with (A) DDM, (B) UDM, (C) DM, and (D) NM.

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# 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<br>B. Trypsin ($M_r$ 23 300) 1 mg/mL<br>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 |

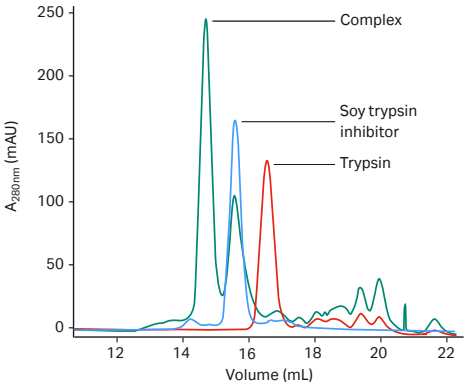



**Fig 11.** Monitoring protein complex formation by analysis on Superdex 200 Increase 10/300 GL. Green chromatogram shows the complex formed between trypsin and soybean trypsin inhibitor. Blue chromatogram shows the soybean trypsin inhibitor peak. Red chromatogram shows the trypsin peak. The complex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

# Ordering information

| Product | Quantity | Product code |
|---|---|---|
| Superdex 200 Increase 10/300 GL | 1 | 28990944 |
| Superdex 200 Increase 5/150 GL | 1 | 28990945 |
| Superdex 200 Increase 3.2/300 | 1 | 28990946 |
| **Related products** | | |
| Gel Filtration LMW Calibration Kit | 1 | 28403841 |
| Gel Filtration HMW Calibration Kit | 1 | 28403842 |
| Superose 6 Increase 10/300 GL | 1 | 29091596 |
| Superose 6 Increase 5/150 GL | 1 | 29091597 |
| Superose 6 Increase 3.2/300 | 1 | 29091598 |
| Superdex 75 Increase 10/300 GL | 1 | 29148721 |
| Superdex 75 Increase 5/150 GL | 1 | 29148722 |
| Superdex 75 Increase 3.2/300 | 1 | 29148723 |
| Superdex 30 Increase 10/300 GL | 1 | 29219757 |
| Superdex 30 Increase 3.2/300 | 1 | 29219758 |
| **Accessories** | | |
| Tricorn™ 10 Filter Kit | 1 | 18115311 |
| Tricorn 5 Filter Kit | 1 | 18115302 |
| Filter Tool | 1 | 18115320 |
| Fingertight connector, 1/16" male | 10 | 18111255 |
| Tricorn storage/shipping device | 1 | 18117643 |
| **Related literature** | | |
| Handbook: Size Exclusion Chromatography, Principles and Methods | | 18102218 |
| Selection Guide: Size Exclusion Chromatography Filtration Columns and Resins | | 18112419 |
| Selection Guide: Prepacked chromatography columns for ÄKTA systems | | 28931778 |

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