Superose™ 6 Increase columns

Superose 6 Increase prepacked columns (Fig 1) are designed for rapid separation and analysis of proteins and other biomolecules by size exclusion chromatography (SEC, also known as gel filtration), in the molecular weight (M_r) range for globular proteins between 5000 and 5 000 000. This very wide fractionation range of the chromatographic medium makes it suitable for purification of protein complexes, membrane proteins, and other macromolecules. The columns are also useful as a screening tool to explore the molecular-weight distribution of unknown samples. This new generation SEC medium replaces the well-known Superose 6 columns.

Superose 6 Increase columns offer:

- Increased resolution compared with Superose 6, for higher purity and improved analysis results.
- Reduced runtime compared with Superose 6, for rapid results.
- Versatile use for both preparative and analytical applications, especially for large proteins and complexes.
- Tolerance to repeated harsh cleaning procedures at high pH, giving long column lifetime and minimal carry-over.

Fig 1. Superose 6 Increase 3.2/300, Superose 6 Increase 10/300 GL, and Superose 6 Increase 5/150 GL prepacked columns are designed for high-resolution SEC of large proteins, protein complexes, and membrane proteins.

Medium characteristics

The chromatography medium in Superose 6 Increase columns is based on a high-flow agarose base matrix with good pressure/flow properties and small bead size. The small beads of the medium allow high-resolution analytical separations. The low nonspecific interaction of the medium permits high recovery of biological materials.

Further characteristics of the Superose 6 Increase medium are shown in Table 1.

Table 1. Main characteristics of Superose 6 Increase medium

Fractionation range	M_r 5000 to 5 × 10^6 (globular proteins) M_p* 1000 to 3 × 10^5 (dextrans)
Exclusion limit (M_r)	Approx. 4 × 10^7 (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
Average bead size	8.6 µm

* Peak molecular mass

Table 2. Characteristics of columns prepacked with Superose 6 Increase

Column	Bed dimensions, diameter × height (mm)	Approximate bed volume (ml)	Column efficiency (theoretical plates m^{-1})	Recommended sample volume (µl)	Recommended flow rate, H_2O at 25°C (ml/min)[1]	Max. flow rate, H_2O at 25°C (ml/min)[1]	Typical pressure drop over column, H_2O at 25°C (MPa)
Superose 6 Increase 10/300 GL	10 × 300	24	> 48 000	25 to 500	0.5	1.5	3.0
Superose 6 Increase 5/150 GL	5 × 150	3	> 42 000	4 to 50	0.3	0.75	3.0
Superose 6 Increase 3.2/300	3.2 × 300	2.4	> 48 000	4 to 50	0.04	0.15	3.0

[1] Note: Flow rate needs to be decreased when working at low temperature or with viscous solutions, see product instructions for more details.

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) When sample amount is limited and small consumption of buffer is important			×
High-resolution analysis (25 to 500 µl)	×		
High-resolution analysis (4 to 50 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		×	

Selectivity of the medium

Both Superdex™ 200 Increase and Superose 6 Increase belong to the new generation of SEC media based on high-flow agarose with small beads. They have different selectivity to complement each other. Figure 2 shows that Superose 6 Increase enables improved separation in the higher molecular weight range compared with Superdex 200 Increase, both for proteins and dextrans.

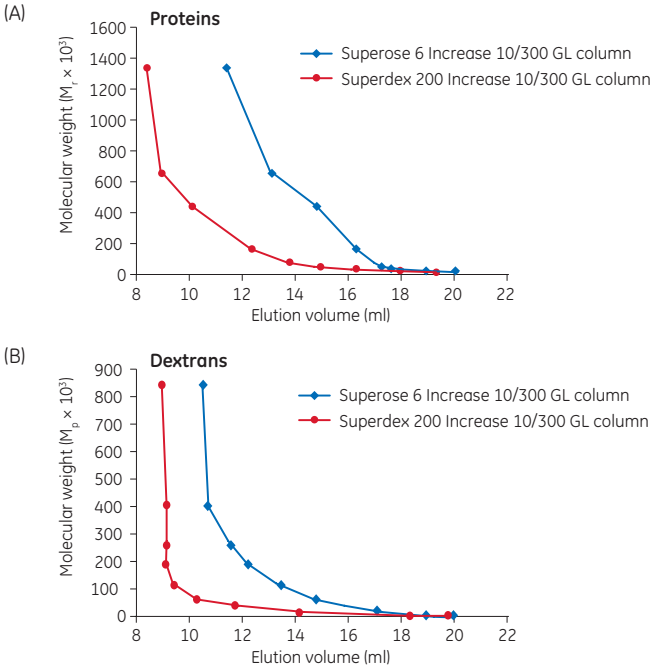


Fig 2. Selectivity of Superose 6 Increase chromatography medium compared with Superdex 200 Increase for a range of (A) proteins from M_r 1 400 000 down to M_r 6500 and (B) dextrans from M_p 850 000 down to M_p 1000.

Chemical stability

Superose 6 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 1%) can be used together with the medium without affecting its performance.

Superose 6 Increase medium also withstands the conditions used for cleaning-in-place (CIP) in the pH range 1 to 14.

Column characteristics and column selection

Superose 6 Increase is available prepacked in Tricorn™ high-performance glass columns of two different sizes (10/300 GL and 5/150 GL) as well as in the column format 3.2/300 (Table 2). All columns are made of glass to allow easier visual inspection of the packed bed. Each column has its own application purpose. Superose 6 Increase 10/300 GL is designed for high-resolution preparative-scale purification (< 500 µl sample volume), protein analysis, and protein characterization. Superose 6 Increase 3.2/300 column is an excellent choice when working with very small sample volumes (4 to 50 µl) and for high-resolution microscale separations using systems such as ÄKTAmicro. Superose 6 Increase 5/150 GL is the first choice for rapid screening, for applications where buffer consumption needs to be low, and for purity checks.

An overview of which column to choose for different applications is shown in Table 3.

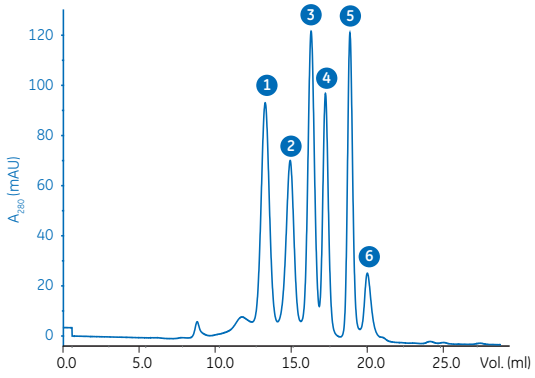
Fittings for simple connection to ÄKTA™ chromatography systems or equivalent high-performance systems are provided. All column parts are biocompatible.

Improved resolution compared with Superose 6

Superose 6 Increase 10/300 GL column has higher resolution capabilities compared with the original Superose 6 10/300 GL column. Figure 3 shows the result of the separation of standard proteins by SEC using Superose 6 Increase 10/300 GL compared with Superose 6 10/300 GL. The sharper peaks observed on Superose 6 Increase confirm higher resolution. The improved resolution can be attributed to the reduced bead size of Superose 6 Increase medium (8.6 µm) compared with the larger beads of Superose 6 (13 µm). The higher rigidity of the chromatography beads and thereby enhanced flow properties enable rapid runs on Superose 6 Increase.

Columns:	Superose 6 Increase 10/300 GL and Superose 6 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. Ovalbumin (M_r 44 000), 3 mg/ml 5. Ribonuclease A (M_r 13 700), 3 mg/ml 6. Aprotinin (M_r 6500), 1 mg/ml
Sample volume:	100 µl
Buffer:	PBS (10 mM phosphate buffer, 140 mM NaCl, pH 7.4)
Flow rate:	0.5 ml/min
System:	ÄKTAmicro

(A) Superose 6 Increase 10/300 GL



(B) Superose 6 10/300 GL

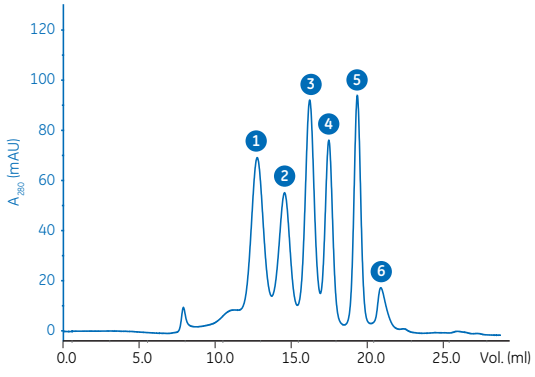


Fig 3. Chromatograms showing high-resolution SEC of six standard proteins on (A) Superose 6 Increase 10/300 GL and (B) Superose 6 10/300 GL.

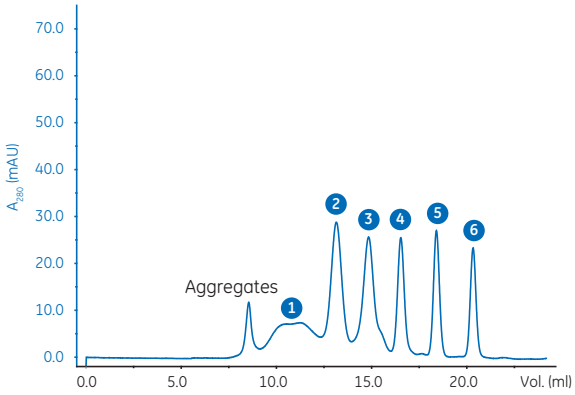
Superose 6 Increase complements Superdex 200 Increase

The high resolution and wide fractionation range of Superose 6 Increase columns allows for effective separation of protein aggregates. In Figure 4, purification of six model proteins on Superose 6 Increase 10/300 GL revealed a broad peak corresponding to IgM. Using Superdex 200 Increase 10/300 GL, this was not observed. It was later confirmed by light scattering that the IgM sample contained isoforms and aggregates.

Columns:	Superose 6 Increase 10/300 GL and Superdex 200 Increase 10/300 GL
Sample:	1. IgM (M_r ~970 000)*, 0.5 mg/ml 2. Thyroglobulin (M_r 669 000), 1 mg/ml 3. Ferritin (M_r 440 000), 0.1 mg/ml 4. BSA (M_r 66 000), 1 mg/ml 5. Myoglobin (M_r 17 000), 0.5 mg/ml 6. Vitamin B_{12} (M_r 1355), 0.05 mg/ml
Sample volume:	100 µl
Buffer:	PBS (10 mM phosphate buffer, 140 mM NaCl, pH 7.4)
Flow rate:	0.5 ml/min
System:	ÄKTAmicro

* Sample also contained aggregated forms of IgM.

(A) Superose 6 Increase 10/300 GL



(B) Superdex 200 Increase 10/300 GL

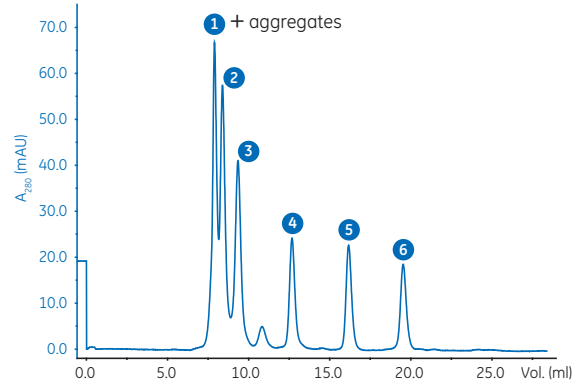


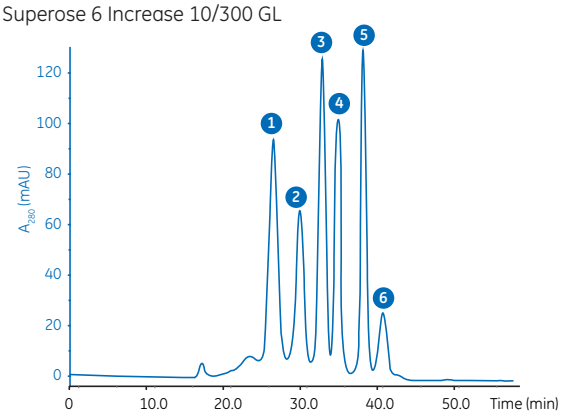
Fig 4. Chromatograms showing high-resolution SEC of six standard proteins on (A) Superose 6 Increase 10/300 GL and (B) Superdex 200 Increase 10/300 GL.

Superose 6 Increase 10/300 GL vs 5/150 GL column: resolution vs speed

Superose 6 Increase 10/300 GL columns are designed for high-resolution SEC at microgram to milligram scale while the smaller 5/150 GL columns are designed for rapid screening. While speed is preferable in applications such as purity checks and size homogeneity analysis, this comes at the cost of decreased resolution. As can be seen in Figure 5A, SEC on the 10/300 GL column of a sample consisting of six proteins gave a high-resolution separation in 50 min. The cycle time on the 5/150 GL column on the other hand was reduced to only 10 min, but the resolution of the separation was also significantly reduced (Fig 5B).

Columns:	Superose 6 Increase 10/300 GL and Superose 6 Increase 5/150 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. Ovalbumin (M_r 44 000), 3 mg/ml 5. Ribonuclease A (M_r 13 700), 3 mg/ml 6. Aprotinin (M_r 6500), 1 mg/ml
Sample volumes:	100 µl, (10/300 GL column) 12 µl, (5/150 GL column)
Buffer:	PBS (10 mM phosphate buffer, 140 mM NaCl, pH 7.4)
Flow rates:	0.5 ml/min (10/300 GL column) 0.3 ml/min (5/150 GL column)
System:	ÄKTAmicro

(A) Superose 6 Increase 10/300 GL



(B) Superose 6 Increase 5/150 GL

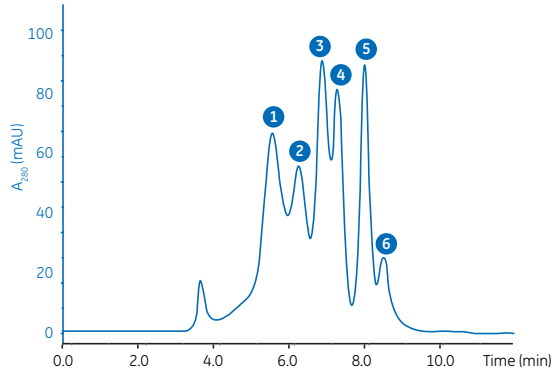


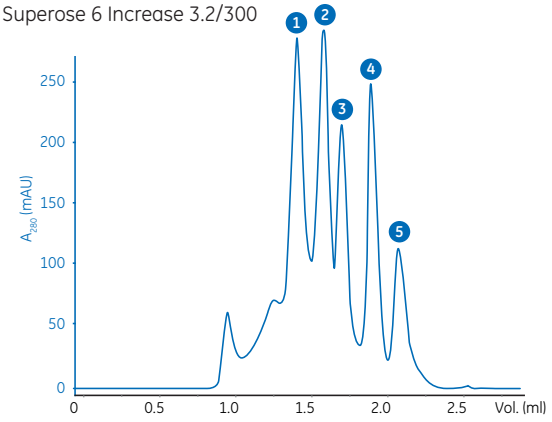
Fig 5. High-resolution SEC of six standard proteins showing relative peak resolution on (A) Superose 6 Increase 10/300 GL compared with (B) the 5/150 GL column.

High sensitivity for small sample volumes

Superose 6 Increase 3.2/300 gives high resolution and good sensitivity for small sample volumes. A 50 µl sample containing five standard proteins was applied both to Superose 6 Increase 3.2/300 and 10/300 GL columns. As shown in Figure 6, the detection signal from the run with Superose 6 Increase 3.2/300 was considerably higher compared with Superose 6 Increase 10/300 GL.

Columns:	Superose 6 Increase 3.2/300 and Superose 6 Increase 10/300 GL
Sample:	1. Thyroglobulin (M_r 669 000), 2 mg/ml 2. Ferritin (M_r 440 000), 0.3 mg/ml 3. Aldolase (M_r 158 000), 3 mg/ml 4. Carbonic anhydrase (M_r 30 000), 1 mg/ml 5. Aprotinin (M_r 6500), 1 mg/ml
Sample volume:	50 µl
Buffer:	PBS (10 mM phosphate buffer, 140 mM NaCl, pH 7.4)
Flow rates:	0.04 ml/min (3.2/300 column) 0.4 ml/min (10/300 GL column)
System:	ÄKTAmicro

(A) Superose 6 Increase 3.2/300



(B) Superose 6 Increase 10/300 GL

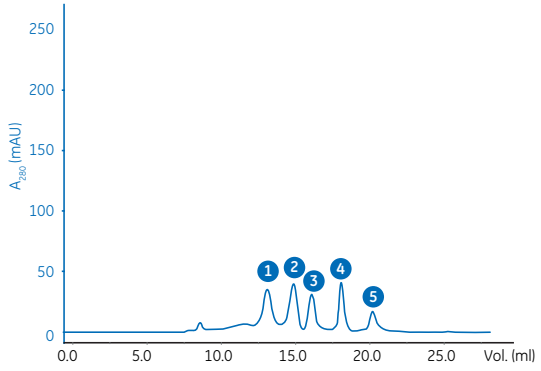


Fig 6. A small volume (50 µl) of a sample mixture containing five proteins was separated on (A) Superose 6 Increase 3.2/300 column and (B) Superose 6 Increase 10/300 GL column. Both chromatograms show the same scale for UV detection on the y-axis.

Toleration of harsh cleaning procedures

The long working life and high reproducibility of Superose 6 Increase prepacked columns are the result of the optimized design, stable nature of the medium, and controlled production procedures. To demonstrate this, a test was performed to verify the chemical stability and robustness of the medium prepacked in Superose 6 Increase 10/300 GL column. Initially, the column was tested with four standard proteins (Fig 7A) followed by a series of injections with 0.6 ml of mouse IgG_{2b} from hybridoma cells. Cleaning-in-place (CIP) with 1.5 CV of 0.5 M NaOH was performed after every 10 injections. After 200 injections with IgG_{2b}, a new test with standard proteins was performed. The results show very similar chromatograms for both the initial run and after 200 injections and 20 CIP cycles (Fig 7).

Column:	Superose 6 Increase 10/300 GL
Sample:	1. Thyroglobulin (M_r 669 000), 2 mg/ml 2. Aldolase (M_r 158 000), 2 mg/ml 3. Ovalbumin (M_r 44 000), 2 mg/ml 4. Ribonuclease A (M_r 13 700), 0.6 mg/ml
Sample volume:	50 µl
Buffer:	PBS (10 mM phosphate buffer, 140 mM NaCl, pH 7.4)
Flow rate:	0.5 ml/min
System:	ÄKTAexplorer 10

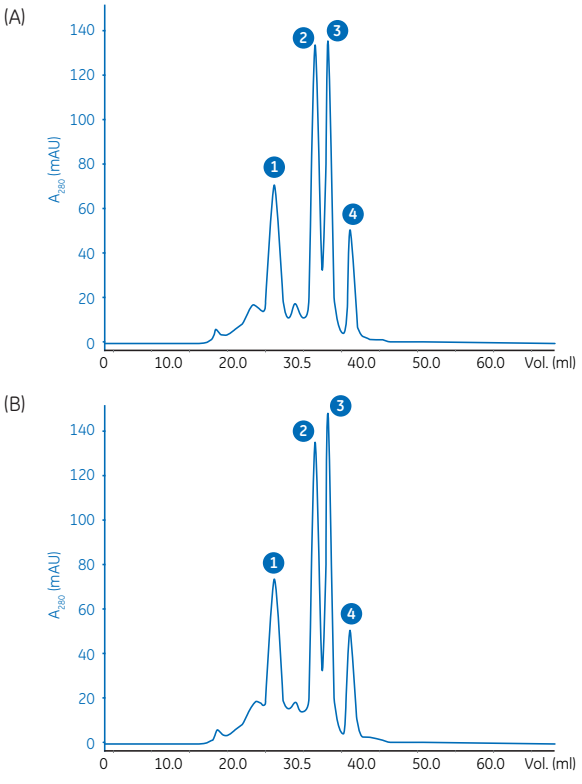


Fig 7. SEC of standard proteins on Superose 6 Increase 10/300 GL (A) before and (B) after 200 injections of IgG_{2b} including 20 CIP cycles with 0.5 M NaOH.

Determination of theoretical plates (N/m) was also performed to evaluate the column efficiency after each of the 20 CIP runs in the experiment described above. Results show that the column efficiency remains high over time (Fig 8) and thus that the medium is chemically stable and withstands high pH.

Column:	Superose 6 Increase 10/300 GL
Sample:	2% acetone in PBS
Sample volume:	50 µl
Buffer:	PBS
Flow rate:	1 ml/min
System:	ÄKTAexplorer 10

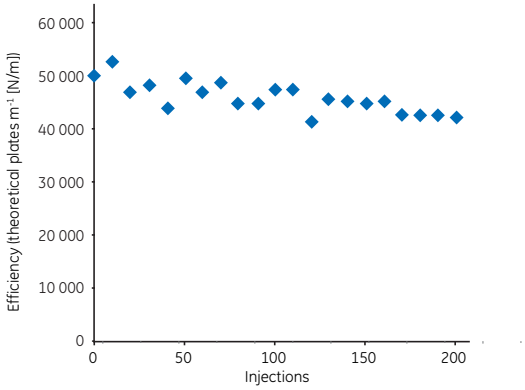


Fig 8. Column efficiency measured in theoretical plates m^{-1} for 200 injections of IgG_{2b} with 20 cycles of CIP.

Operation

Choice of eluents

An eluent that ensures the sample is fully soluble and, if possible, that will simplify downstream applications, should be selected. Chaotropic agents and detergents can be used to improve the solubility of membrane proteins. Since ionic interactions can occur with both acidic and basic proteins at very low salt concentrations, it is recommended to include 150 mM to 300 mM NaCl or to use a buffer with equivalent ionic strength to minimize these interactions.

Sample volumes and flow rates

When working with protein concentrations below 10 mg/ml, SEC 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, resolution can be increased. For more information, see Table 2.

To protect the packed bed from compression, it is important to decrease flow rate when working at low temperature or with viscous solutions, see instructions for use for more details.

Applications

Oligomerization of a pathogenic protein involved in neurodegenerative disorders

Lewy bodies are abnormal inclusions found inside nerve cells in patients with Parkinson's disease and related neurodegenerative disorders. These protein abnormalities constitute mainly aggregated forms of the protein α-synuclein. Aldehydes such as 4-oxo-2-nonenal (ONE) formed during oxidative stress are believed to be involved in the formation of Lewy bodies.

Monomeric α-synuclein was incubated for 24 h with ONE at a ratio of 30:1 (ONE: α-synuclein) and the material was separated on Superose 6 Increase 3.2/300. Figure 9A shows the peak of monomeric α-synuclein. The resulting chromatogram after incubation with ONE shows that monomeric α-synuclein has been oligomerized and no monomeric form can be detected (Fig 9B). The oligomerized material was further used for *in-vitro* and *in-vivo* studies of its neurotoxicity.

Column:	Superose 6 Increase 3.2/300
Sample:	(A) Monomeric α-synuclein; (B) Monomeric α-synuclein incubated for 24 h with the aldehyde, ONE
Sample volume:	10 µl
Buffer:	TBS (50 mM Tris, 150 mM NaCl, pH 7.4)
Flow rate:	50 µl/min
System:	Merck™ Hitachi™ D-7000 LaChrom

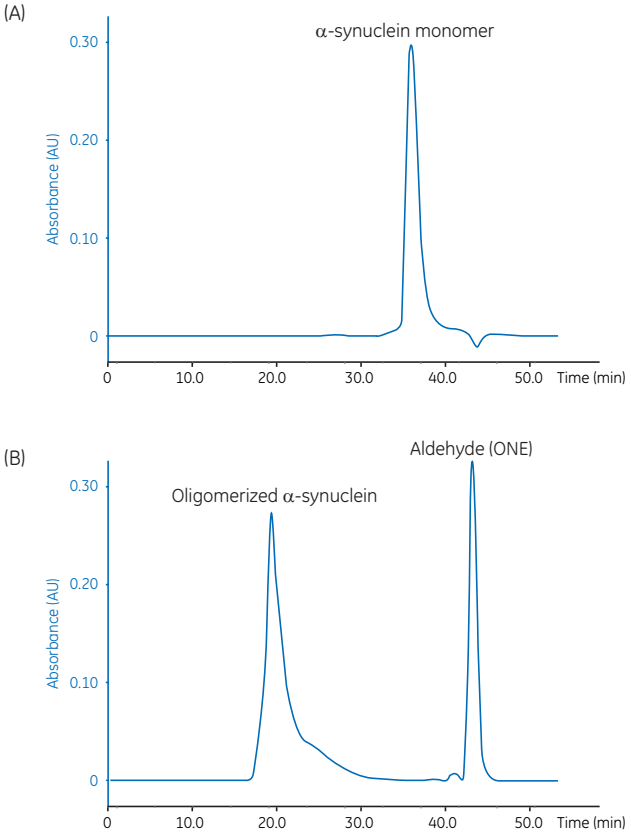


Fig 9. SEC on Superose 6 Increase 3.2/300 of (A) monomeric α-synuclein and (B) monomeric α-synuclein after incubation with the aldehyde, ONE. Data courtesy of Dr. Joakim Bergström, Rudbeck Laboratory, Uppsala University.

Purification of a membrane protein complex for structure and molecular mechanism studies

ATP synthetase complex from *E. coli* membrane was captured with Ni Sepharose™ 6 Fast Flow and further purified on Superose 6 Increase 5/150 GL. As seen in Figure 10, the resolution between aggregates (peak 1) and monomer ATP synthetase protein complex was good (peak 2), as well as separation from degradation products (peaks 3 and 4). In addition to small consumption of sample and buffer, the separation was achieved with low runtime, yet high resolution. The purified material was further used for structure and molecular mechanism studies.

Column:	Superose 6 Increase 5/150 GL
Sample:	ATP synthetase complex from *E. coli* membrane
Sample volume:	25 µl
Buffer:	20 mM Tris-HCl, 150 mM NaCl, 10% glycerol, 250 µM $MgCl_2$, 0.05% dodecyl maltoside, pH 8.0
Flow rate:	0.15 ml/min
System:	ÄKTAmicro in cold room

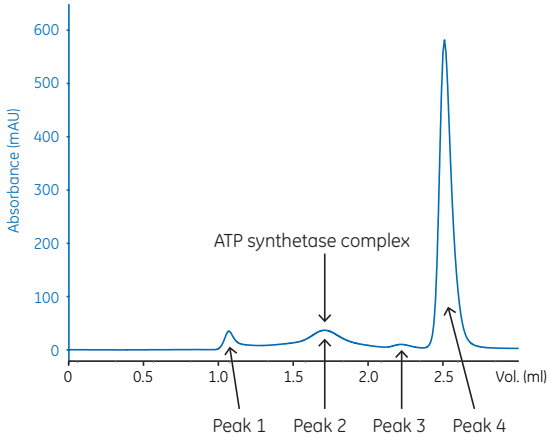


Fig 10. SEC using Superose 6 Increase 5/150 GL of ATP synthetase complex from *E. coli* membrane. Peak 1: Aggregates; Peak 2: Monomer ATP synthetase complex; Peak 3 and 4: Degradation products.

Acknowledgements

We thank Dr. Joakim Bergström, Rudbeck Laboratory, Uppsala University for the oligomerization of α-synuclein study.

Ordering information

Product	Quantity	Code number
Superose 6 Increase 10/300 GL	1	29-0915-96
Superose 6 Increase 5/150 GL	1	29-0915-97
Superose 6 Increase 3.2/300	1	29-0915-98

Related products

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

Accessories

Tricorn 10 Filter Kit[1]	1	29-0536-12
Tricorn 5 Filter Kit[1]	1	29-0535-86
Filter Tool	1	18-1153-20
Fingertight connector, 1/16” male	10	18-1112-55
Tricorn storage/shipping device	1	18-1176-43

[1] Do not store exposed to daylight

Related literature

Handbook: Size-exclusion chromatography, principles and methods	18-1022-18
Selection guide: Prepacked chromatography columns for ÄKTA systems	28-9317-78
Instrument management handbook: ÄKTA laboratory-scale chromatography systems	29-0108-31

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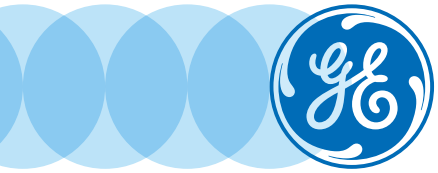

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