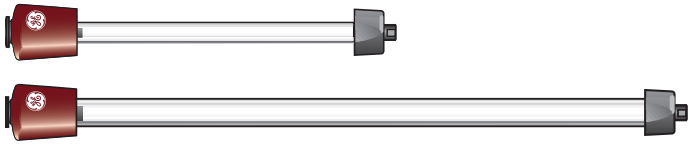
# 29-0915-97 Superose™ 6 Increase 5/150 GL

# 29-0915-96 Superose 6 Increase 10/300 GL



Please read these instructions carefully before using the columns.

### Intended use

Superose 6 Increase 5/150 GL and Superose 6 Increase 10/300 GL columns are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

### Safety

For use and handling of the products in a safe way, please refer to the Safety Data Sheet.

## Quick information

Superose 6 Increase 5/150 GL and Superose 6 Increase 10/300 GL are pre-packed Tricorn™ glass columns.

Superose 6 Increase 5/150 GL is for rapid size analysis of proteins and other biomolecules. Short cycle time, together with small sample volume and low consumption of buffer make this column ideal for use in screening experiments to check protein homogeneity.

Superose 6 Increase 10/300 GL is suitable for small scale preparative purification (µg-mg) as a final polishing step, as well as for protein analysis and characterization.

The columns are supplied with two fingertight connectors 1/16" male for connection to ÄKTA™ or other systems.

**Table 1. Medium data**

| | |
|---|---|
| Matrix | Composite of cross-linked agarose |
| Average particle size | 8.6 µm |
| Exclusion limit ($M_r$) | Approx. $4 \times 10^7$ |
| Optimum separation range | |
| globular proteins ($M_r$) | 5000 to $5 \times 10^6$ |
| dextrans ($M_p$) | 1000 to $3 \times 10^5$ |
| pH stability range | |
| regular use | 3 to 12 |
| cleaning | 1 to 14 |
| Temperature | |
| operating | 4°C to 40°C |
| storage | 4°C to 30°C |

**Table 2. Column data**

| | 5/150 | 10/300 |
|---|---|---|
| Bed dimensions (mm) | 5 × 153-158 | 10 × 300-310 |
| Approximate bed volume (ml) | 3 | 24 |
| Column efficiency (N/m) | > 42 000 | > 48 000 |
| Typical pressure drop over packed bed[1] | 3.0 MPa[2], 30 bar, 435 psi | 3.0 MPa[2], 30 bar, 435 psi |
| Column hardware pressure limit | 10 MPa, 100 bar, 1450 psi | 5.0 MPa, 50 bar, 725 psi |

[1] Determine the limit according to section *Setting column pressure limits.*

[2] At maximum flow rate at 25° in water.

**Table 3. Flow rate limits and recommendations Superose 6 Increase 5/150 GL**

| Temperature | | Flow rate (ml/min) |
|---|---|---|
| 20°C to 25°C | Maximum flow rate, water | 0.75 |
| | Recommended flow rate, water | 0.30 |
| | Maximum flow rate, 20% ethanol | 0.35 |
| 4°C to 8°C | Maximum flow rate, water | 0.35 |
| | Maximum flow rate, 20% ethanol | 0.15 |

**Table 4. Flow rate limits and recommendations Superose 6 Increase 10/300 GL**

| Temperature | | Flow rate (ml/min) |
|---|---|---|
| 20°C to 25°C | Maximum flow rate, water | 1.5 |
| | Recommended flow rate, water | 0.5 |
| | Maximum flow rate, 20% ethanol | 0.75 |
| 4°C to 8°C | Maximum flow rate, water | 0.75 |
| | Maximum flow rate, 20% ethanol | 0.35 |

## First time use

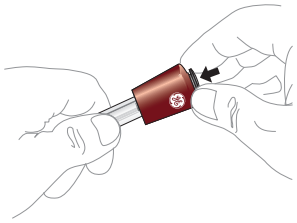


**Fig 1.** Illustration of how to lock the adapter. The locking ring (black) must be in the down-position to prevent uncontrolled adjustment of the column's bed height.

Before connecting the column to a chromatography system, make sure there is no air in the tubing and valves. Remove the storage/shipping device, see section *Delivery/storage*, and the stop plug from the column. Check that the upper adapter is locked (locking ring pressed down, see Fig. 1). Make sure that the column inlet is filled with liquid and connect it drop-to-drop to the system. For maximum resolution especially on the 5/150 column, minimize dead volumes between the injection valve and the column as well as between the column outlet and the detector.

Prepare the column for first-time use as follows:

a) Equilibrate with at least 2 column volumes (CV) of room tempered water at a flow rate of 0.25 ml/min (5/150) or 0.5 ml/min (10/300).

b) Set pressure limits for the column in your method according to section *Setting column pressure limits*.

c) Equilibrate with at least 2 CV eluent at a flow rate of 0.25 ml/min (5/150) or 0.5 ml/min (10/300).

d) It is recommended to perform a column performance control for future comparisons, see section *Column performance control*.

**NOTICE**
Make sure not to exceed the pressure limits of the column. This is particularly important when working at low temperatures, like in a cold room, or when the column is used with 20% ethanol or other viscous solutions. Set pressure limits according to section *Setting column pressure limits*.

## Setting column pressure limits

There are two pressure limits to consider when running the column, the pressure drop over the packed bed and the column hardware pressure limit. The pressure drop over the packed bed differ for each column and the limit has to be individually set. Note that it might be significantly lower compared to the maximum value noted in Table 2.

Exceeding any pressure limit may lead to collapse of the gel bed or damage to the column hardware. Increased pressure is for example generated when running/using one or a combination of the following parameters:

- Eluent or sample with high viscosity compared to water
- Low temperature compared to room temperature
- Modifications to the flow path, for example changing to thinner/longer tubing

For optimal functionality it is important to know the pressure drops over different parts of your system and how they affect the column. All ÄKTA chromatography systems measure pressure at the system pump, $\mathbf{p_{pump}}$ (see Fig. 2). Some systems have additional pressure sensors located before and after the column, $\mathbf{p_{pre\text{-}cp}}$ and $\mathbf{p_{post\text{-}cp}}$.

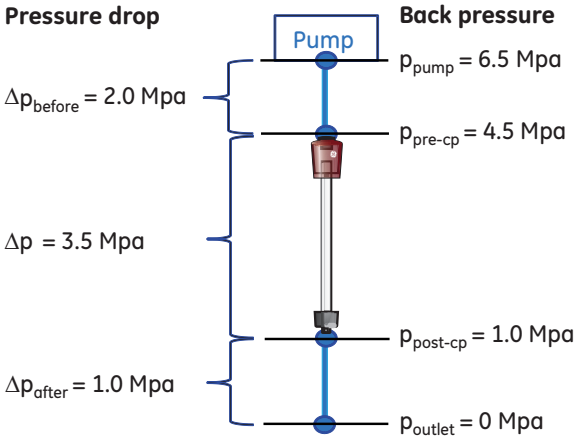



**Fig 2.** Example of the pressure in different parts of a system during run of a column.

- $\Delta\mathbf{p_{before}}$ does not affect the column.
- The pressure on the column hardware is the sum of $\Delta\mathbf{p_{after}}$ and $\Delta\mathbf{p}$. Do not exceed the column hardware limit!
- $\Delta\mathbf{p}$, is individual for each column and needs to be determined.

For more information, please refer to the *ÄKTA laboratory-scale Chromatography Systems Instrument Management Handbook*.

### How to set pressure limit for ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC, ÄKTAmicro, ÄKTA pure (Column Valve V9-Cs) and other systems with a pressure sensor in the pump

Determination of column specific pressure drop over the packed bed, $\Delta\mathbf{p}$ (see Fig. 2):

1 $\Delta\mathbf{p}_{\mathbf{before}}$ is measured in absence of the column. Run the pump at maximum flow rate of the column with water at room temperature (see Table 3 or Table 4). Let the flow drip from the tubing that will later be connected to the column. Note the pressure as $\Delta\mathbf{p}_{\mathbf{before}}$.

2 Connect the column equilibrated with room tempered water to the system. Run the pump at the same flow rate as in step 1, let the flow drip from the column outlet. Note the pressure value.

3 $\Delta\mathbf{p}$ is calculated as the pressure value in step 2 minus $\Delta\mathbf{p}_{\mathbf{before}}$. The $\Delta\mathbf{p}$ value will be used in step 7 below.

This $\Delta\mathbf{p}$ should not be exceeded at any temperature or using any liquid.

Setting pressure limit in method at your experimental conditions (intended system setup, flow rate, temperature and eluent):

4 $\Delta\mathbf{p}_{\mathbf{before}}$ is measured in absence of the column. Run the pump at your intended flow rate. Let the flow drip from the tubing that will later be connected to the column. Note the pressure as $\Delta\mathbf{p}_{\mathbf{before}}$.

5 Instead of the column, connect a piece of tubing[1] to the system. Run the pump at the same conditions as in step 4. Note the pressure value as the total system pressure.

6 $\Delta\mathbf{p}_{\mathbf{after}}$ is calculated as the total system pressure value noted in step 5 minus $\Delta\mathbf{p}_{\mathbf{before}}$, noted in step 4.

7 Calculate $\Delta\mathbf{p} + \Delta\mathbf{p}_{\mathbf{after}}$. If this value is lower than the column hardware pressure limit (see Table 2), set the pressure limit in your method as $\Delta\mathbf{p}+\Delta\mathbf{p}_{\mathbf{after}}+\Delta\mathbf{p}_{\mathbf{before}}$.

You can now start your experiment!

If $\Delta\mathbf{p} + \Delta\mathbf{p}_{\mathbf{after}}$ exceed the column hardware pressure limit, reduce the flow rate or $\Delta\mathbf{p}_{\mathbf{after}}$. Repeat step 4-7.

[1]Avoid thin and/or long tubing that will give back pressure.

### How to set pressure limit for ÄKTA avant and ÄKTA pure (Column Valve V9-C)

$\Delta\mathbf{p}$ and $\mathbf{p}_{\mathbf{pre\text{-}cp}}$ (see Fig. 2) are automatically monitored by the system. Note that the measured values include the tubing used to connect the column to the instrument.

Setting pressure limit in method:

1 Check that the **max pre column pressure alarm** is set to the same as the column hardware pressure limit (see Table 2).

2 Connect the column equilibrated with room tempered water to the system. Start running the column at a low flow rate (e.g., half recommended flow rate) with water/eluent at room temperature. Slowly increase the flow until the maximum flow rate of the column or the limit set in step 1 is reached (see Table 3 or Table 4). Note the pressure over the packed bed, $\Delta\mathbf{p}$, (DeltaC pressure) and set the value as **max delta column pressure**.

This $\Delta\mathbf{p}$ should not be exceeded at any temperature or using any liquid.

You can now start your experiment!

## Column performance control

In order to detect any changes in column performance, it is very important that you make an initial test with your particular system configuration. Note that the contribution from dead volumes in the instrument to band broadening will vary depending on system set-up and will influence column efficiency. The obtained efficiency on your system might be lower compared to the specifications in Table 2.

### Column efficiency test

Column efficiency, expressed as the number of theoretical plates per meter, N/m, is calculated using the following equation:

$$N/m = 5.54 \times (V_R / W_h)^2 / L$$

where

| | | |
|---|---|---|
| N/m | = | number of theoretical plates/meter |
| $V_R$ | = | volume eluted from the start of sample application to the peak maximum |
| $W_h$ | = | peak width measured as the width of the recorded peak at half of the peak height |
| L | = | bed height (m) |

***Column Superose 6 Increase 5/150 GL***

Check the performance of the column using the following procedure:

| | |
|---|---|
| Sample: | 10 µl 2% acetone (20 mg/ml) in buffer or water |
| Eluent: | Buffer or water |
| Flow rate: | 0.30 ml/min, room temperature |
| Detection: | 280 nm |

***Column Superose 6 Increase 10/300 GL***

Check the performance of the column using the following procedure:

| | |
|---|---|
| Sample: | 100 µl 2% acetone (20 mg/ml) in buffer or water |
| Eluent: | Buffer or water |
| Flow rate: | 1.0 ml/min, room temperature |
| Detection: | 280 nm |

## Function test

As an alternative to the above efficiency test, check the column performance by running a function test.

***Column 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. Carbonic anhydrase ($M_r$ 29 000) 1 mg/ml |
| | 5. Aprotinin ($M_r$ 6500) 1 mg/ml |
| Sample volume: | 12 µl |
| Eluent: | 0.01 M phosphate buffer, 0.14 M NaCl, pH 7.4 |
| Flow rate: | 0.3 ml/min, room temperature |
| Detection: | 280 nm |

Result is shown in Fig. 3.

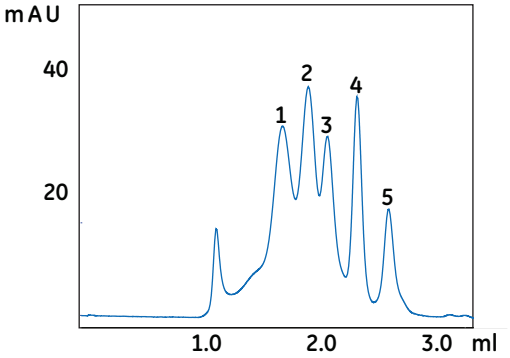



**Fig 3.** Typical chromatogram from a function test of Superose 6 Increase 5/150 GL using ÄKTAmicro.

***Column Superose 6 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. 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 |
| Eluent: | 0.01 M phosphate buffer, 0.14 M NaCl, pH 7.4 |
| Flow rate: | 0.5 ml/min, room temperature |
| Detection: | 280 nm |

Result is shown in Fig. 4.

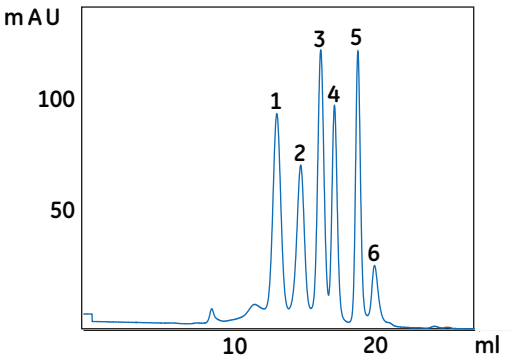



**Fig 4.** Typical chromatogram from a function test of Superose 6 Increase 10/300 GL using ÄKTAmicro.

## Try these conditions first

| | |
|---|---|
| Eluent: | 0.01 M phosphate buffer, 0.14 M NaCl, pH 7.4 |
| Flow rate: (room temperature) | 0.3 ml/min (5/150); 0.5 ml/min (10/300) |
| Sample volume: | 10 µl (5/150); 100 µl (10/300) |

Equilibration is not necessary between runs with the same buffer. Read the section *Optimization* for information on how to optimize a separation.

## Sample recommendations

| | |
|---|---|
| Molecular weight ($M_r$): | 5000 to $5 \times 10^6$ |
| Protein concentration: | Up to 50 mg/ml, for higher resolution below 10 mg/ml. |
| Sample volume: | 4 to 50 µl (5/150)<br>25 to 500 µl (10/300) |
| Preparation: | Dissolve the sample in eluent, filter through a 0.22 µm filter or centrifuge at 10 000 g for 10 min. |

## System recommendations

The small bed volume of the 5/150 column makes it sensitive to dead volumes in the system. For this column it is recommended to use systems like ÄKTApurifier 10, ÄKTA pure 25, or ÄKTAmicro. Use short, narrow capillaries and avoid unnecessary components in the flow path. ÄKTA start is not compatible with Superose 6 Increase columns due to low maximum operating pressure.

***Note:*** *Be aware of pressure limits.*

The 10/300 column has a larger bed volume and can be used in systems like ÄKTAexplorer 10 and ÄKTA avant 25 in addition to the systems mentioned above. ÄKTA start is not compatible with Superose 6 Increase columns due to low maximum operating pressure.

***Note:*** *Be aware of pressure limits.*

***Superose 6 Increase 5/150 GL column on different systems***

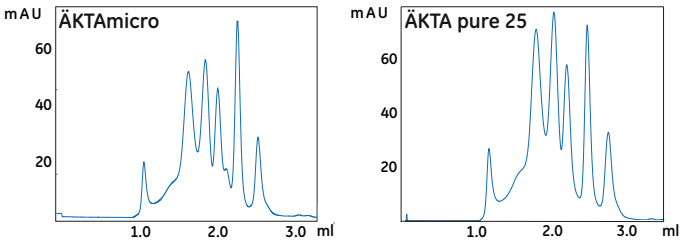



**Fig 5.** Comparison of protein separation on Superose 6 Increase 5/150 GL on different systems. Due to different UV cells for the systems, mAU scales differ.

***Superose 6 Increase 10/300 GL column on different systems***

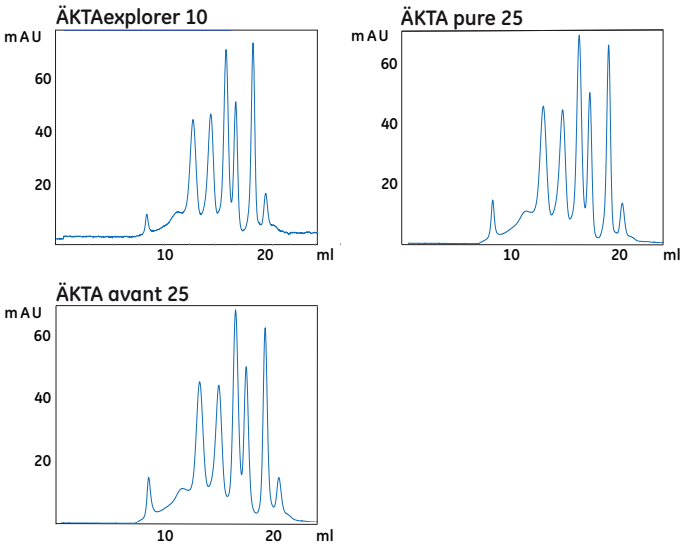



**Fig 6.** Comparison of protein separation on Superose 6 Increase 10/300 GL on different systems. Due to different UV cells for the systems, mAU scales differ.

## Delivery/storage

The column is delivered with a storage/shipping device that prevents it from drying out. The column is equilibrated with degassed 20% ethanol.

If the column is to be stored for more than 2 days after use, wash the column with 2 CV water and then equilibrate with at least 2 CV 20% ethanol.

***Note:*** *Use a lower flow rate for 20% ethanol. See Table 3 and Table 4.*

We recommend that you connect the storage/shipping device according to section *How to connect the storage/shipping device* for long term storage.


***How to remove the storage/shipping device***

1 Push down the spring-loaded cap.

2 Remove the locking pin.

3 Release the cap and unscrew the device.

***How to refill the storage/shipping device***

1 Connect a syringe or pump to the storage/shipping device and fill with 20% ethanol over the mark on the tube. Remove the syringe or connection to the pump.

2 Tap out air bubbles and push the plunger to the mark on the device.


***How to connect the storage/shipping device***

1 Fill the column inlet and luer connector with 20% ethanol and connect the filled storage/shipping device drop-to-drop to the top of the column.

2 Mount the spring-loaded cap (2) and secure it with the locking pin (3).

The glass tube is coated with a protecting plastic film. Small quantities of air may occasionally be trapped between the glass and the film during manufacturing. The resulting uneven surface does not affect column performance or durability.

## Choice of eluent

Select an eluent that ensures the sample is fully soluble. Also try to choose an eluent that will simplify downstream applications. For example, if the proteins/peptides are to be lyophilized, a volatile eluent is necessary. Since ionic interactions can occur with both acidic and basic proteins at very low salt concentrations, a recommended buffer is 0.01 to 0.05 M sodium phosphate with additional 0.15 to 0.3 M NaCl, pH 7.4. Table 5 lists some useful eluent compositions.

**Table 5. Useful eluent compositions**

| pH | Buffer/eluent | Properties/application examples |
|---|---|---|
| 5.0 | 0.1 M ammonium acetate | Good solubility for some enzymes, e.g., cellulases. Volatile. |
| 6.8 | 0.2 M sodium phosphate | Suitable for some antibody separations. |
| 7.2 | 0.05 M phosphate + 0.15 M NaCl | Physiological conditions. |
| 7.8 | 0.15 M ammonium hydrogen carbonate | Suitable for some DNA and protein separations. Volatile. Should be used fresh. |
| 8.0 | 0.1 M Tris-HCl, 0.001 M EDTA | Very good solubility for DNA and RNA. |
| 8.6 | 6 M guanidine hydrochloride in 0.05 M Tris-HCl | Good UV-transparency. Suitable if there is a need to purify proteins under denaturing conditions. |
| 11.5 | 0.05 M NaOH | Good solubility for some compounds. |

| Buffer additives | Properties/application examples |
|---|---|
| Up to 8 M urea (pH<7) | Good solubility for many components. Biological activity can be maintained at lower urea contents. Certain risk for carbamylation of proteins. |
| 6 M guanidine hydrochloride | Molecular weight determinations of subunits. |
| 0.1% SDS, Tween or similar | Good solubility for some proteins, e.g., membrane proteins. Make sure you equilibrate completely with the detergent solution. |
| 10% acetonitrile | For separation of hydrophobic compounds. Volatile. |
| 0.2 M arginine | Decreases tendency of aggregation. |

## Buffers and solvent resistance

De-gas and filter all solutions through a 0.22 µm filter. Install an on-line filter before the injection valve.

***Note:*** *Buffers and solvents with increased viscosity will affect the back pressure. Reduce the flow rate if necessary.*

### Long term use

Long term use refers to use where the medium is stable over a long period of time without adverse side effects on its chromatographic performance.

- All commonly used aqueous buffers, pH 3 to 12
- Urea, up to 8 M
- Ionic and non-ionic detergents, e.g., 1% SDS
- Guanidine hydrochloride, up to 6 M
- Isopropanol, up to 5%
- Methanol, up to 10%
- Sodium hydroxide, up to 0.5 M
- Dithiothreitol, up to 5 mM

### Short term use

Short term use refers to the use during regeneration, cleaning-in-place, and sanitization procedures.

- Acetonitrile, up to 30%
- Sodium hydroxide, up to 1 M
- Ethanol, up to 70%
- Methanol, up to 100%
- Acetic acid, up to 1 M
- Isopropanol, up to 30%
- Hydrochloric acid, up to 0.1 M
- Trifluoroacetic acid, up to 10%
- Formic acid, up to 70%

### Avoid:

- Oxidizing agents
- Unfiltered solutions

## Optimization

If your results are unsatisfactory, consider the following actions.

### Flow rate

**Action:** Decrease the flow rate.

**Effect:** Improves resolution for high molecular weight components. The resolution for small components may be decreased.

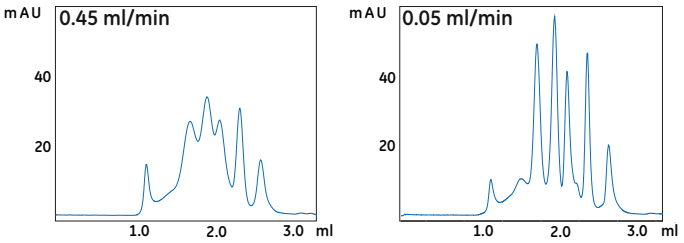



**Fig 7.** Comparison of protein separation on Superose 6 Increase 5/150 GL at different flow rates.

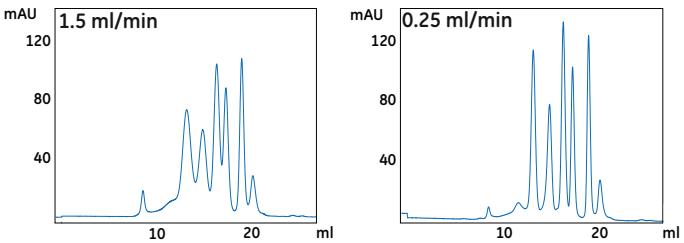



**Fig 8.** Comparison of protein separation on Superose 6 Increase 10/300 GL at different flow rates.

### Sample volume

**Action:** Decrease the sample volume.

**Effect:** Improves resolution.

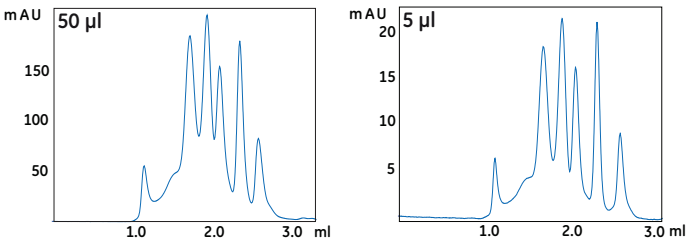



**Fig 9.** Comparison of protein separation on Superose 6 Increase 5/150 GL using different sample volumes.

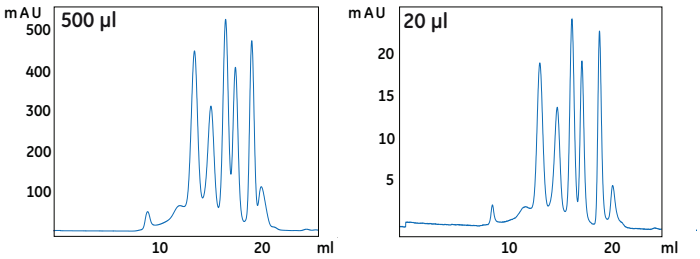



**Fig 10.** Comparison of protein separation on Superose 6 Increase 10/300 GL using different sample volumes.

### System dead volumes

**Action:** Decrease system dead volumes.

**Effect:** Improves resolution.

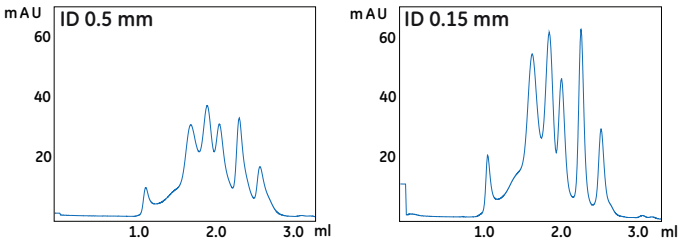



**Fig 11.** Comparison of protein separation on Superose 6 Increase 5/150 GL using different diameters of a 32 cm long capillary connected to the column.

For more information, please refer to the handbook *Gel filtration, Principles & Methods*.

## Cleaning-in-place (CIP)

Perform the following regular cleaning cycle after 10 to 20 separation cycles. Increasing pressure drop over the packed bed indicate that the column needs to be cleaned.

***Note:*** *When performing CIP, reversed flow is recommended.*

### Regular cleaning

1 Wash the column with 1 CV 0.5 M sodium hydroxide alternatively 0.5 M acetic acid at a flow rate of 0.1 ml/min (5/150) or 0.5 ml/min (10/300).

2 Immediately rinse the column with 1 CV water followed by at least 2 CV eluent at a flow rate of 0.1 ml/min (5/150) or 0.5 ml/min (10/300).

Before the next run, equilibrate the column until the UV baseline and pH are stable. Check that the column performance has been restored according to section *Column performance control*.

### More rigorous cleaning

- Depending on the nature of the contaminants, the cleaning solutions in section *Buffers and solvent resistance* may be used. Always rinse with at least 2 CV water after any of the cleaning solutions have been used.
- If column performance is not restored, wash the column with 3 CV 0.5 M arginine. Rinse with at least 2 CV water.
- If column performance is still not restored, inject a solution of 1 mg/ml pepsin in 0.1 M acetic acid containing 0.5 M NaCl and leave overnight at room temperature or one hour at 37°C. After enzymatic treatment, clean the column according to the procedure described in the section *Regular cleaning*.
- Change the filter at the top of the column. (Since contaminants are introduced with the liquid flow, many of them are caught by the filter). Instructions for changing the filter are supplied with the Filter Kit. Perform a regular cleaning as described above.
- If necessary, suspend 2 to 3 mm of the top of the gel bed and remove it with a Pasteur pipette. Adjust the adaptor to eliminate the space above the gel.

## Troubleshooting

| Symptom | Remedy |
|---|---|
| Increased back-pressure over the column and/or loss of resolution. | Confirm that the column is the cause (see below). If so, clean it according to the procedure described in section *Cleaning in place (CIP).* |
| | To confirm that the high back-pressure in the system is caused by the column, disconnect one piece of equipment at a time (starting at the fraction collector) with the pumps running. Check the pressure reading after each piece has been disconnected to determine the source of the back-pressure. |
| Air in the column | Note that small amounts of air will normally not affect the performance of the column. Run 3 to 4 CV well de-gassed eluent in an upflow direction at a flow rate of 0.2 ml/min (5/150) or 0.5 ml/min (10/300) at room temperature. |
| Space between gel bed and adapter | Turn down the adapter to the gel bed. Perform a column performance control. |

## Ordering information

| Product | Quantity | Code No. |
|---|---|---|
| Superose 6 Increase 5/150 GL | 1 | 29-0915-97 |
| Superose 6 Increase 10/300 GL | 1 | 29-0915-96 |

### Related products

| Product | Quantity | Code No. |
|---|---|---|
| Superose 6 Increase 3.2/300 | 1 | 29-0915-98 |
| Superdex™ 200 Increase 3.2/300 | 1 | 28-9909-46 |
| Superdex 200 Increase 5/150 GL | 1 | 28-9909-45 |
| Superdex 200 Increase 10/300 GL | 1 | 28-9909-44 |
| Gel filtration LMW Calibration Kit | 1 | 28-4038-41 |
| Gel filtration HMW Calibration Kit | 1 | 28-4038-42 |

### Accessories

| Product | Quantity | Code No. |
|---|---|---|
| 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.

### Literature

| Handbook | Code No. |
|---|---|
| Gel filtration Principles & Methods | 18-1022-18 |
| ÄKTA laboratory-scale Chromatography Systems Instrument Management Handbook | 29-0108-31 |

For local office contact information, visit
www.gelifesciences.com/contact

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