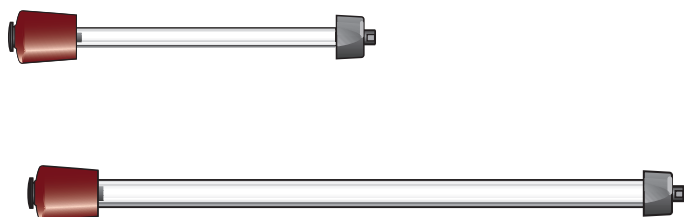


# Superdex 75 Increase 5/150 GL

# Superdex 75 Increase 10/300 GL

## Size exclusion chromatography columns

## Instructions for Use



Read these instructions carefully before using the columns.

### Intended use

Superdex™ 75 Increase 5/150 GL and Superdex 75 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, refer to the Safety Data Sheet.

### Quick information

Superdex 75 Increase 5/150 GL and Superdex 75 Increase 10/300 GL are prepacked Tricorn™ glass columns. The glass tube is coated with a protecting plastic film.

Superdex 75 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 purity, size-homogeneity, stability, etc.

Superdex 75 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.** Resin data

Matrix	Composite of cross-linked agarose and dextran
Particle size, $d_{50V}$ <sup>1</sup>	~ 9 µm
Exclusion limit, globular proteins ( $M_r$ )	Approx. $1 \times 10^5$
Fractionation range	
globular proteins ( $M_r$ )	3 000 to 70 000
dextran ( $M_p$ )	500 to 30 000
pH stability range	
operational <sup>2</sup>	3 to 12
CIP <sup>3</sup>	1 to 14
Temperature	
operational	4°C to 40°C
storage	4°C to 30°C

<sup>1</sup> Median particle size of the cumulative volume distribution.

<sup>2</sup> pH range where resin can be operated without significant change in function.

<sup>3</sup> pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

**Table 2.** Column data

	<b>5/150</b>	<b>10/300</b>
Bed dimensions (mm)	5 x 153-158	10 x 300-310
Approximate bed volume (mL)	3	24
Column efficiency (N/m)	> 38 000	> 43 000
Typical pressure drop over packed bed <sup>1</sup>	3.0 MPa <sup>2</sup> , (30 bar, 435 psi)	3.0 MPa <sup>2</sup> , (30 bar, 435 psi)
Column hardware pressure limit	10 MPa, (100 bar, 1450 psi)	5 MPa, (50 bar, 725 psi)

<sup>1</sup> Determine the limit according to section Setting column pressure limits.

<sup>2</sup> At maximum flow rate at 25°C in water.

**Table 3.** Flow rate limits and recommendations Superdex 75 Increase 5/150 GL

Temperature		Flow rate (mL/min)
20°C to 25°C	Recommended flow rate, water	0.45
	Maximum flow rate, water	0.75
	Maximum flow rate, 20% ethanol	0.35
	Maximum flow rate, 10% glycerol	0.35
4°C to 8°C	Maximum flow rate, water	0.35
	Maximum flow rate, 20% ethanol	0.17
	Maximum flow rate, 10% glycerol	0.17

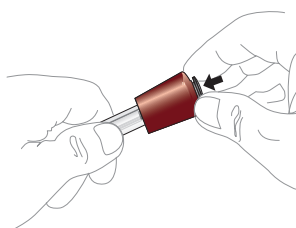
**Table 4.** Flow rate limits and recommendations Superdex 75 Increase 10/300 GL

Temperature		Flow rate (mL/min)
20°C to 25°C	Recommended flow rate, water	0.80
	Maximum flow rate, water	1.60
	Maximum flow rate, 20% ethanol	0.80
	Maximum flow rate, 10% glycerol	0.80
4°C to 8°C	Maximum flow rate, water	0.80
	Maximum flow rate, 20% ethanol	0.40
	Maximum flow rate, 10% glycerol	0.40

**Note:** Most water-based buffers can be considered to be similar to water, for example phosphate and Tris buffers.

**Note:** When running viscous samples (for example containing glycerol) it is important to lower the flow rate, see recommendations above.

## 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 [Delivery/storage, on page 5](#), and the stop plug from the column. Check that the upper adapter is locked (locking ring pressed down, see Figure above). 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 all dead volumes between the injection valve and the column as well as between the column outlet and the detector.

**Note:** Buffer solution, column and system should be kept at the same temperature when operating.

Prepare the column for first-time use as follows:

Step	Action
1	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). Be aware to lower the flow rate if run at lower temperature.
2	Set pressure limits for the column in your method according to section Setting column pressure limits.
3	Equilibrate with at least 2 CV eluent at a flow rate of 0.45 mL/min (5/150) or 0.8 mL/min (10/300).
4	It is recommended to perform a column performance control for future comparisons, see <a href="#">Column performance control, on page 4</a> .



### 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. Decrease the flow rate according to [Table 3, on page 2](#) or [Table 4, on page 2](#). Set pressure limits according to [Setting column pressure limits, on page 2](#).

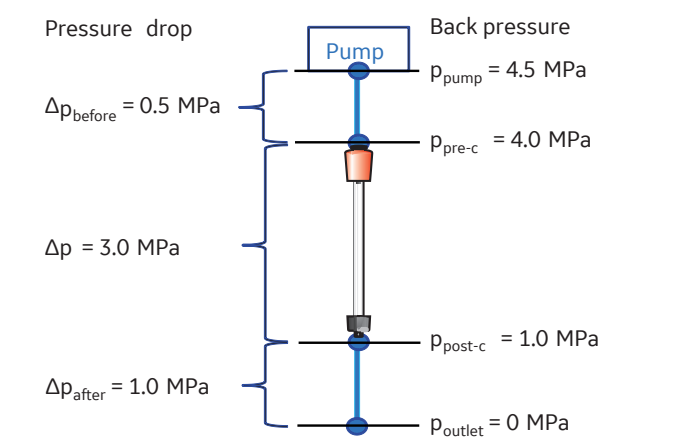
## Setting column pressure limits

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 differs for each column and the limit has to be individually set as the columns are packed to withstand the maximum flow rate. Note that it might be different compared to the value noted in [Table 2, on page 1](#).

Exceeding any pressure limit may lead to collapse of the column bed or damage to the column hardware. Increased pressure may for example be generated by one or a combination of the following parameters:

- Eluent or sample with high viscosity compared to water. This includes 20% ethanol.
- Low temperature compared to room temperature.
- Modifications to the flow path, for example changing to thinner or 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,  $p_{\text{pump}}$  (see Figure below). Some systems have additional pressure sensors located before and after the column,  $p_{\text{pre-c}}$  and  $p_{\text{post-c}}$ .



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

- $\Delta p_{\text{before}}$  does not affect the column.
- The pressure on the column hardware is the sum of  $\Delta p_{\text{after}}$  and  $\Delta p$  (on some systems monitored separately as  $p_{\text{pre-c}}$ ). Do not exceed the column hardware limit!
- $\Delta p$  is individual for each column and needs to be determined.

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

### How to set pressure limit for ÄKTAexplorer, ÄKTApurifier, ÄKTAmicro, and other systems with a pressure sensor in the pump

Determination of column specific pressure drop over the packed bed,  $\Delta p$  (see Figure above):

Step	Action
1	$\Delta p_{\text{before}}$ is measured in absence of the column. Run the pump at maximum flow rate of the column in water and at the temperature for the experimental conditions. For exact values, see <a href="#">Table 3, on page 2</a> or <a href="#">Table 4, on page 2</a> . Let the flow drip from the tubing that will later be connected to the column. Note the pressure as $\Delta p_{\text{before}}$ .
2	Check that the Pressure Alarm in software is set to the same as the Column hardware pressure limit.
3	Connect the column to the system. Let the flow drip from the column outlet. The column should be equilibrated in water and at the temperature for the experimental conditions. Run the pump at the same flow rate as in step 1. Note the pressure value.
4	$\Delta p$ is calculated as the pressure value in step 3 minus $\Delta p_{\text{before}}$ . The $\Delta p$ value will be used in step 8 below.

<sup>1</sup> Avoid thin and/or long tubing that will give back pressure.

Step	Action
	This $\Delta p$ 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):
5	$\Delta p_{\text{before}}$ 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 $\Delta p_{\text{before}}$ .
6	Instead of the column, connect a piece of tubing <sup>1</sup> to the system. Run the pump at the same conditions as in step 5. Note the pressure value as the total system pressure.
7	$\Delta p_{\text{after}}$ is calculated as the total system pressure value noted in step 6 minus $\Delta p_{\text{before}}$ , noted in step 5.
8	Calculate $\Delta p + \Delta p_{\text{after}} + \Delta p_{\text{before}}$ <ul style="list-style-type: none"> <li>• If this value is lower than the Column hardware pressure limit (see <a href="#">Table 2, on page 1</a>), set the pressure limit in your method as <math>\Delta p + \Delta p_{\text{after}} + \Delta p_{\text{before}}</math>.</li> <li>• If <math>\Delta p + \Delta p_{\text{after}}</math> exceeds the column hardware pressure limit, reduce the flow rate or <math>\Delta p_{\text{after}}</math>. Repeat step 5-8.</li> </ul>

You can now start your experiment!

### How to set pressure limit for ÄKTA pure, without a column valve or with Column Valve V9-Cs (1 column)

$p_{\text{pre-c}}$  (see Figure above) is automatically monitored by the system. This is the pressure signal to use in the following instruction. Do not use the System pressure signal. Note that the measured values include the tubing used to connect the column to the instrument.

Determination of column specific pressure drop over the packed bed ( $\Delta p$ ):

Step	Action
1	Check that the Alarm pre column pressure in software is set to the same as the Column hardware pressure limit (see <a href="#">Table 2, on page 1</a> ).
2	Connect the column to the system. Let the flow drip from the column outlet. The column should be equilibrated in water and at the temperature for the experimental conditions. Run the pump at maximum flow rate of the column in water and at the temperature for the experimental conditions. For exact values, see <a href="#">Table 3, on page 2</a> or <a href="#">Table 4, on page 2</a> .  Note the pressure value. The now measured $p_{\text{pre-c}}$ value is the maximum pressure over the packed bed, $\Delta p$ (DeltaC pressure).
3	This $\Delta p$ value should not be exceeded at any temperature or with any liquid!
	Setting pressure limit in method at your experimental conditions (intended system setup, flow rate, temperature and eluent):
4	$\Delta p_{\text{after}}$ is measured in absence of the column. Run the pump at your intended flow rate.
5	Instead of the column, connect a piece of tubing <sup>1</sup> to the system, or bypass the column if connected to a valve. Run the pump at your intended flow rate. The now measured value is $\Delta p_{\text{after}}$ .

Step	Action
6	Calculate $\Delta p + \Delta p_{\text{after}}$ . <ul style="list-style-type: none"> <li>If this value is lower than the Column hardware pressure limit (see <a href="#">Table 2, on page 1</a>), set the pressure limit in your method, Alarm pre column pressure, as <math>\Delta p + \Delta p_{\text{after}}</math></li> <li>If <math>\Delta p + \Delta p_{\text{after}}</math> exceeds the column hardware pressure limit, reduce the flow rate or <math>\Delta p_{\text{after}}</math>. Repeat step 4-6.</li> </ul>

You can now start your experiment!

### How to set pressure limit for ÄKTA avant and ÄKTA pure with Column Valve V9-C (5 columns)

**Note:** This is not an optimal high performance setup. See [System recommendations, on page 5](#) and [System dead volumes, on page 7](#) for more details.

$\Delta p$  and  $p_{\text{pre-c}}$  (see [Fig. 2, on page 3](#)) are automatically monitored by the system.

**Note:** The measured values include the tubing used to connect the column to the instrument.

Step	Action
1	Check that the Alarm pre column pressure is set to the same as the Column hardware pressure limit (see <a href="#">Table 2, on page 1</a> ).
2	Connect the column to the system. The column should be equilibrated in water and at the temperature for the experimental conditions. Start running the column at a low flow rate. Slowly increase the flow until the maximum flow rate for the column under these conditions or the limit set in step 1 is reached. For exact maximum flow rates under different conditions, see <a href="#">Table 3, on page 2</a> or <a href="#">Table 4, on page 2</a> . Note the pressure over the packed bed, $\Delta p$ (delta-column pressure), and set the value as Alarm Delta column pressure.  This $\Delta p$ should not be exceeded at any temperature or with 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 column 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, on page 1](#)

### Column efficiency test

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

$$N/m = 5.54 \times (V_R / W_h)^2 / L, \text{ where}$$

$N/m$	=	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 Superdex 75 Increase 5/150 GL

Check the performance of the column using the following procedure:

Sample:	10 $\mu$ L 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 Superdex 75 Increase 10/300 GL

Check the performance of the column using the following procedure:

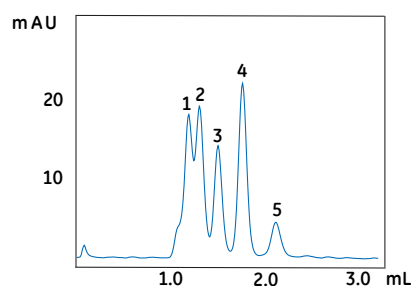
Sample:	100 $\mu$ L 2% acetone (20 mg/mL) in buffer or water
Eluent:	Buffer or water
Flow rate:	1.0 mL/min, room temperature
Detection:	280 nm

### Function test

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

### Column Superdex 75 Increase 5/150 GL

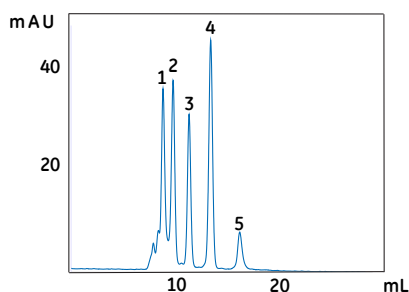
Sample:	<ol style="list-style-type: none"> <li>1. Conalbumin (<math>M_r</math> 75 000) 1.5 mg/mL</li> <li>2. Ovalbumin (<math>M_r</math> 44 000) 4 mg/mL</li> <li>3. Carbonic anhydrase (<math>M_r</math> 29 000) 1.5 mg/mL</li> <li>4. Ribonuclease A (<math>M_r</math> 13 700) 3 mg/mL</li> <li>5. Aprotinin (<math>M_r</math> 6 500) 1 mg/mL</li> </ol> (All from Gel Filtration LMW Calibration Kit)
System	ÄKTA pure 25
Sample volume:	10 $\mu$ L
Eluent:	PBS (0.01 M phosphate buffer, 0.14 M NaCl, 0.003 M KCl, pH 7.4)
Flow rate:	0.45 mL/min, room temperature
Detection:	280 nm



**Fig 3.** Typical chromatogram from a function test of Superdex 75 Increase 5/150 GL.

## Column Superdex 75 Increase 10/300 GL

Sample:	<ol style="list-style-type: none"> <li>1. Conalbumin (<math>M_r</math> 75 000) 1.5 mg/mL</li> <li>2. Ovalbumin (<math>M_r</math> 44 000) 4 mg/mL</li> <li>3. Carbonic anhydrase (<math>M_r</math> 29 000) 1.5 mg/mL</li> <li>4. Ribonuclease A (<math>M_r</math> 13 700) 3 mg/mL</li> <li>5. Aprotinin (<math>M_r</math> 6 500) 1 mg/mL</li> </ol> (All from Gel Filtration LMW Calibration Kit)
System	ÄKTA pure 25
Sample volume:	100 $\mu$ L
Eluent:	PBS (0.01 M phosphate buffer, 0.14 M NaCl, 0.003 M KCl, pH 7.4)
Flow rate:	0.8 mL/min, room temperature
Detection:	280 nm



**Fig 4.** Typical chromatogram from a function test of Superdex 75 Increase 10/300 GL.

## Try these conditions first

Eluent:	PBS (0.01 M phosphate buffer, 0.14 M NaCl, 0.003 M KCl, pH 7.4)
Flow rate:	0.45 mL/min (5/150); 0.80 mL/min (10/300) (room temperature)
Sample volume:	10 $\mu$ L (5/150); 100 $\mu$ L (10/300)

Equilibration is not necessary between runs with the same buffer. Read the section [Optimization, on page 7](#) for information on how to optimize a separation.

## Sample recommendations

Molecular weight ( $M_r$ ):	3 000 to 70 000
Protein concentration:	Up to 50 mg/mL, for higher resolution below 10 mg/mL.
Sample volume:	4 to 50 $\mu$ L (5/150) 25 to 500 $\mu$ L (10/300)
Preparation:	The sample should be fully soluble in the eluent used. Filter through a 0.22 $\mu$ m filter or centrifuge at 10 000 g for 10 min.

**Note:** High sample viscosity (high protein concentration or additives) can cause instability of the separation and the back pressure might increase. Dilute sample or decrease flow rate during sample application.

## System recommendations

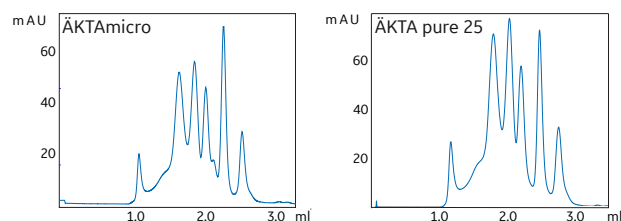
The small bed volume of the 5/150 column makes it sensitive to dead volumes in the system. For this column it is recommended to use systems like ÄKTApurifier 10, ÄKTA pure 25 without the column valve V9-C (5 columns), ÄKTA micro, or similar. Use short, narrow capillaries and avoid all unnecessary components in the flow path. For optimal configuration of ÄKTA pure 25, see Cue Cards in the Literature list in [Ordering information, on page 8](#).

**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. Consider actions to reduce system dead volumes if sufficient performance is not achieved.

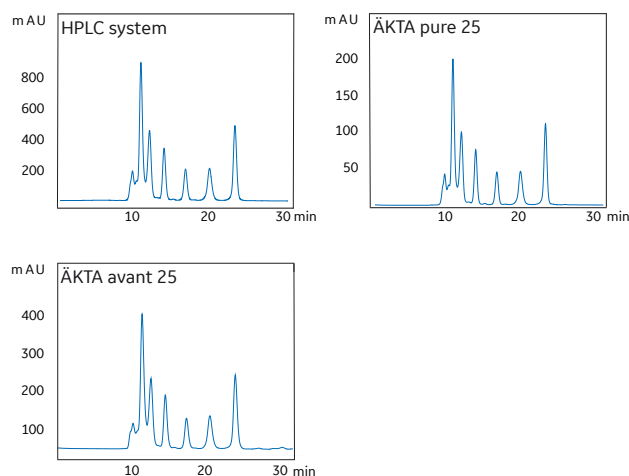
ÄKTA start is not compatible with Superdex 75 Increase columns due to too low maximum operating pressure.

## Superdex 75 Increase 5/150 GL on different systems



**Fig 5.** Comparison of protein separation on Superdex 75 Increase 5/150 on different systems, an HPLC system and ÄKTA pure 25.

## Superdex 75 Increase 10/300 GL on different systems



**Fig 6.** Comparison of protein separation on Superdex 75 Increase 10/300 on different systems, an HPLC system, ÄKTA pure 25 and ÄKTA avant 25.

## 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 column volumes (CV) of water and then equilibrate with at least 2 CV 20% ethanol.

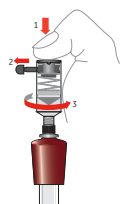
**Note:** Use a lower flow rate for 20% ethanol.

For long term storage, we recommend that you connect the storage/shipping device according to [How to connect the storage/shipping device, on page 6](#) for long term storage.

## 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 <a href="#">Cleaning-in-place (CIP), on page 8</a> .  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 column volumes / (CV) of 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. (Longer adapters than the original ones are available, see <a href="#">Ordering information, on page 8</a> .) Perform a Column performance control.

### 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 NaCl, pH 7.4. Table (below) 5 lists some useful eluent compositions.

#### 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	0.01-0.05 M phosphate + 0.15 M NaCl	Near 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.

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. Thoroughly remove the detergent with, e.g., 70% ethanol before doing separations without detergent.
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. See [Table 3, on page 2](#) and [Table 4, on page 2](#).

### Long term use

Long term use refers to use where the resin 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%
- Dithiothreitol (DTT), up to 5 mM
- Acetic acid, up to 1M
- Sodium hydroxide, up to 0.5 M
- Methanol, up to 10%
- Acetonitrile, up to 10%

### 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 70%
- Isopropanol, up to 30%
- Hydrochloric acid, up to 0.1 M
- Trifluoroacetic acid, up to 1%
- Formic acid, up to 70%

### Avoid:

- Oxidizing agents
- Non-filtered samples and eluents

## Optimization

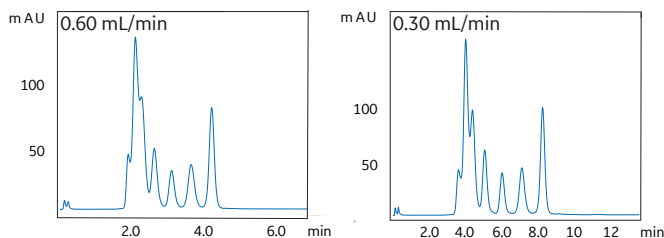
If your results are unsatisfactory, consider the following actions.

For more information, see also the Literature list in [Ordering information, on page 8](#).

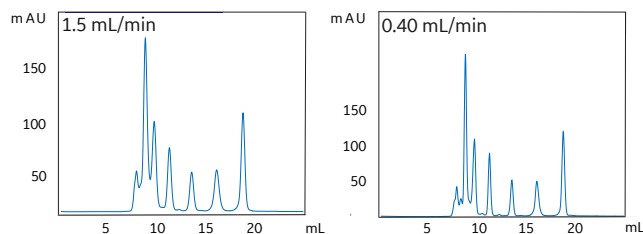
### Flow rate

**Action:** Decrease the flow rate.

**Effect:** Generally improves resolution.



**Fig 7.** Comparison of protein separation on Superdex 75 Increase 5/150 at different flow rates.

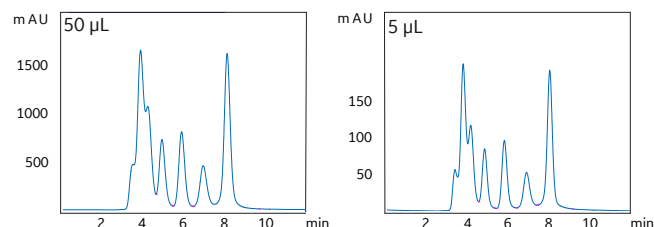


**Fig 8.** Comparison of protein separation on Superdex 75 Increase 10/300 at different flow rates.

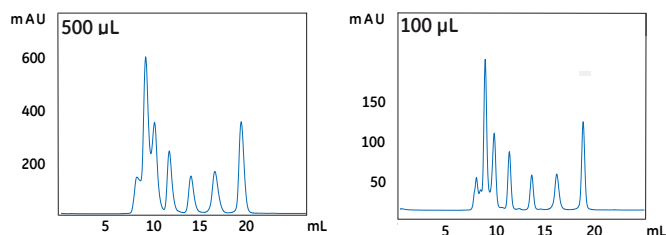
### Sample volume

**Action:** Decrease the sample volume.

**Effect:** Improves resolution.



**Fig 9.** Comparison of protein separation on Superdex 75 Increase 5/150 using different sample volumes.



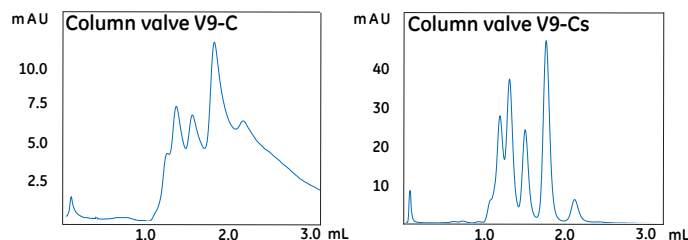
**Fig 10.** Comparison of protein separation on Superdex 75 Increase 10/300 using different sample volumes

### System dead volumes

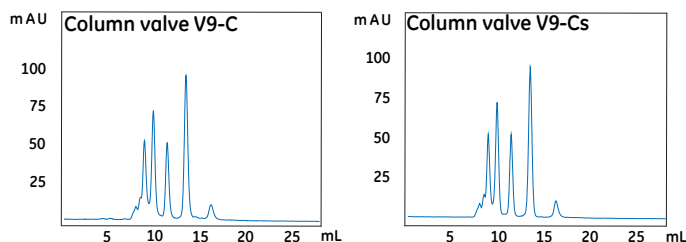
**Action:** Decrease system dead volumes e.g., by using a suitable column valve (or no valve) and using short, narrow-diameter capillaries.

**Effect:** Improves resolution.

Note that in both comparison examples below, separations without any column valve gave no or very little resolution improvement (not shown). Be aware that column bypass is not available without a column valve. For further information, see Cue Cards in the Literature list in [Ordering information, on page 8](#).



**Fig 11.** Comparison of protein separation on Superdex 75 Increase 5/150 with ÄKTA pure 25 using either column valve V9-C or column valve V9-Cs. Note that column valve V9-C is not recommended to use with 5/150 columns.



**Fig 12.** Comparison of protein separation on Superdex 75 Increase 10/300 with ÄKTA pure 25 using either column valve V9-C or column valve V9-Cs.

For a comparison example of the effect of different diameters of capillaries: See the Instruction for Superdex 200 Increase (Instruction 29027271). See also the Literature list in [Ordering information, on page 8](#).

## Organic solvent

**Action:** Add organic solvent.

**Effect:** Increases solubility of hydrophobic compounds. Changes selectivity.

## Columns in tandem

**Action:** Connect two columns in series.

**Effect:** Increases resolution due to increased bed height. Back pressure will increase. Be sure not to exceed maximum pressure limits. See comparison example in Instructions for Superdex 200 Increase (Instruction 29027271).

For more information, see also the Literature list in [Ordering information, on page 8](#).

## Cleaning-in-place (CIP)

Perform the following regular cleaning cycle after 10 to 20 separation cycles, or when otherwise needed.

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

**Note:** For storage after CIP procedure be sure to rinse with buffer back to neutral pH before transferring to 20% ethanol.

## Regular cleaning

Step	Action
1	Wash the column with 1 column volume (CV) of 0.5 M sodium hydroxide alternatively 0.5 M acetic acid at a flow rate of 0.13 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 of buffering eluent at a flow rate of 0.13 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 the column according to Section Column performance control that the performance is restored.

## More rigorous cleaning

- Depending on the nature of the contaminants, one of the cleaning solutions in the Section Buffers and solvent resistance may be used. Always rinse with at least 2 CV water after any of the cleaning solution has been used, followed by 2 CV of buffering eluent.
- 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, fill the column with 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, carefully suspend 2 to 3 mm of the top of the gel bed and remove it with a Pasteur pipette. Adjust the adapter to eliminate the space above the gel. (Longer adapters than the original ones are available, see [Ordering information, on page 8](#).)

## Ordering information

Product	Quantity	Product code
Superdex 75 Increase 5/150 GL	1	29148722
Superdex 75 Increase 10/300 GL	1	29148721

## Related products

Product	Quantity	Product code
Superdex 75 Increase 3.2/300 GL	1	29148723
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 30 Increase 10/300 GL	1	29219757
Superdex 30 Increase 3.2/300	1	29219758
Superdex 200 Increase 10/300 GL	1	28990944
Superdex 200 Increase 5/150 GL	1	28990945
Superdex 200 Increase 3.2/300	1	28990946
Gel filtration LMW Calibration Kit	1	28403841
Gel filtration HMW Calibration Kit	1	28403842

## Accessories

Product	Quantity	Product code
Tricorn 10 Filter Kit	1	29053612
Tricorn 5 Filter Kit	1	29053586
Filter tool	1	18115320
Fingertight connector, 1/16" male	10	18111255
Tricorn storage/shipping device	1	18117643
(Long) Tricorn 5 adapter unit	1	28406406
(Long) Tricorn 10 adapter unit	1	28406407

## Literature

Document	Product code
Size Exclusion Chromatography, Principles & Methods	18102218
ÄKTA laboratory-scale Chromatography	29010831
Systems Instrument Management Handbook	
Procedure: Maintenance and cleaning of size exclusion chromatography columns	29140760
Cue Cards: Optimal configuration of ÄKTA pure 25 for small scale SEC	29181181



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