29219758 **Superdex** 30 Increase 3.2/300

Instructions for Use

Read these instructions carefully before using the column.

Intended use

Superdex™ 30 Increase 3.2/300 column is 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 product in a safe way, refer to the Safety Data Sheet.

Quick information

Superdex 30 Increase 3.2/300 is a prepacked high performance glass column. The glass tube is protected with a plastic outer shell. This column is intended for sensitive and high resolving size exclusion chromatography of polypeptides and other biomolecules in the micro preparative scale and for analytical purposes.

The column is supplied with two fingertight connectors 1/16" male for connection to ÄKTA™ or other systems. The column cannot be opened or refilled.

Table 1. Resin data

Matrix		Composite of cross-linked agarose and dextran
Particle size, d_{50v} [1]		~ 9 µm
Fractionation range (M_r)		
	peptides	100 to 7000
pH stability range		
	operational[2]	3 to 12
	CIP[3]	1 to 14
Temperature		
	operational	4°C to 40°C
	storage	4°C to 30°C

[1] Median particle size of the cumulative volume distribution.

[2] pH range where resin can be operated without significant change in function.

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

Table 2. Column data

Bed dimensions (mm)	3.2 × 300
Approximate bed volume (mL)	2.4
Column efficiency (N/m)	> 38 000
Typical pressure drop over packed bed[1]	2.0 MPa[2] (20 bar, 290 psi)
Column hardware pressure limit	5 MPa (50 bar, 725 psi)

[1] Determine the limit according to section *Setting column pressure limits, on page 2*.

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

Table 3. Flow rate limits and recommendations

Temperature		Flow rate (mL/min)
20°C to 25°C	Recommended flow rate, water	0.075
	Maximum flow rate, water	0.150
	Maximum flow rate, 20% ethanol	0.075
	Maximum flow rate, 10% glycerol	0.075
	Maximum flow rate, 30% acetonitrile	0.150
	Maximum flow rate, 40% methanol	0.075
4°C to 8°C	Maximum flow rate, water	0.075
	Maximum flow rate, 20% ethanol	0.035
	Maximum flow rate, 10% glycerol	0.035
	Maximum flow rate, 30% acetonitrile	0.075
	Maximum flow rate, 40% methanol	0.035

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

Before connecting the column to a chromatography system, make sure there is no air in the tubing and valves. Remove the storage/shipping device and the stop plug from the column, see section *Delivery/storage, on page 4*. Make sure that the column inlet is filled with liquid and connect it drop-to-drop to the system. For maximum resolution on the 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 0.04 mL/min. Be aware to lower the flow rate if run at lower temperature.
2	Determine the column specific maximum pressure according to the following section.
3	Equilibrate with at least 2 CV eluent at a flow rate of 0.075 mL/min.
4	It is recommended to perform a column performance control for future comparisons. See section *Column performance control, on page 3*.

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 the following section.

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 as the column is 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 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. This includes 20% ethanol.
- 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 the following figure). Some systems have additional pressure sensors located before and after the column, $\mathbf{p_{pre\text{-}cp}}$ and $\mathbf{p_{post\text{-}cp}}$.

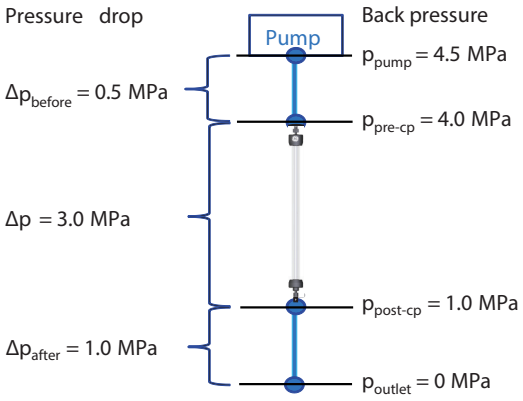


Fig 1. Example of the pressure in different parts of a system during run of a column.

- $\mathbf{\Delta p_{before}}$ does not affect the column.
- The pressure on the column hardware is the sum of $\mathbf{\Delta p_{after}}$ and **Δp**. Do not exceed the column hardware limit!
- **Δp**, 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 ÄKTA explorer, ÄKTA purifier, ÄKTA micro, and other systems with a pressure sensor in the pump

Determination of column specific pressure drop over the packed bed, Ip (see the figure above):

Step	Action
1	$\mathbf{\Delta p_{before}}$ 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 *Table 3, on page 1*. Let the flow drip from the tubing that will later be connected to the column. Note the pressure as $\mathbf{\Delta p_{before}}$.
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	**Δp** is calculated as the pressure value in step 3 minus $\mathbf{\Delta p_{before}}$. The **Δp** value will be used in step 8 below. This **Δp** 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	$\mathbf{\Delta p_{before}}$ 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 $\mathbf{\Delta p_{before}}$.

Step	Action
6	Instead of the column, connect a piece of tubing[1] to the system. Run the pump at the same conditions as in step 5. Note the pressure value as the total system pressure.
7	Δp_{after} is calculated as the total system pressure value noted in step 6 minus Δp_{before}, noted in step 5.
8	Calculate $\Delta p + \Delta p_{after.} + \Delta p_{before}$
	a. If this value is lower than the Column hardware pressure limit (see *Table 2, on page 1*), set the pressure limit in your method as $\Delta p + \Delta p_{after.} + \Delta p_{before.}$
	b. If $\Delta p + \Delta p_{after}$ exceeds the column hardware pressure limit, reduce the flow rate or $\Delta p_{after.}$
	Repeat step 5-8.

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_{pre-c} (see *Fig. 1, on page 2*) 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 (Δp):

Step	Action
1	Check that the Alarm pre column pressure in software is set to the same as the Column hardware pressure limit (see *Table 2, on page 1*).
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 *Table 3, on page 1*.
	Note the pressure value.
	The now measured p_{pre-c} value is the maximum pressure over the packed bed, Δp (*DeltaC pressure*).
3	This Δp 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):

Step	Action
4	Δp_{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[2] 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 Δp_{after}.
6	Calculate $\Delta p + \Delta p_{after}$.
	a. If this value is lower than the Column hardware pressure limit (see *Table 2, on page 1*), set the pressure limit in your method, Alarm pre column pressure, as $\Delta p + \Delta p_{after}$.
	b. If $\Delta p + \Delta p_{after}$ exceeds the column hardware pressure limit, reduce the flow rate or Δp_{after}.
	Repeat step 4-6.

You can now start your experiment.

Column Valve V9-C for ÄKTA pure and ÄKTA avant

Note: *It is not recommended to use Valve V9-C due to large dead volume.*

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, thus 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:

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

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)

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:	0.1 mL/min, room temperature
Detection:	280 nm

Function test

As an alternative to the above efficiency test, check the column performance by running the function test shown in *Fig. 2, on page 4*.

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

Sample:	1. Cytochrome C (Mr 12 400) 0.2 mg/mL 2. Aprotinin (Mr 6500) 0.2 mg/mL 3. Vitamin B12 (Mr 1355) 0.07 mg/mL 4. Triglycine (Mr 189) 0.2 mg/mL 5. Glycine (Mr 75) 7 mg/mL The proteins/peptides were diluted in 2×PBS
System	HPLC system
Sample volume:	10 µL
Eluent:	2×PBS (0.02 M phosphate buffer, 0.28 M NaCl, 0.006 M KCl, pH 7.4)
Flow rate:	0.075 mL/min, room temperature
Detection:	214 nm

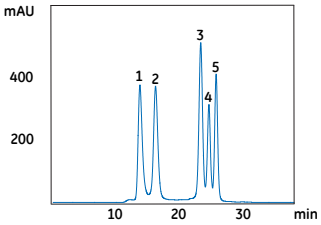


Fig 2. Typical chromatogram from a function test of Superdex 30 Increase 3.2/300.

Try these conditions first

Eluent:	2×PBS (0.02 M phosphate buffer, 0.28 M NaCl, 0.006 M KCl, pH 7.4)
Flow rate:	0.075 mL/min
(room temperature)	
Sample volume:	10 µL

Equilibration is not necessary between runs with the same eluent buffer. Read the Section *Optimization, on page 6* for information on how to optimize a separation.

Sample recommendations

Molecular weight (M_r):	100 to 7000
Protein concentration:	Up to 10 mg/mL
Sample volume:	4 to 50 µL
Preparation:	The sample should be fully soluble in the eluent used. Filter through a 0.22 µm 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 3.2/300 column makes it sensitive to dead volumes in the system. It is recommended to use systems like ÄKTAmicro, ÄKTApurifier 10, ÄKTA pure 25, and HPLC systems. Use short, narrow capillaries and avoid all unnecessary components in the flow path. Valve V9C (5 columns) is not recommended in the flow path due to large internal volume. For optimal configuration of ÄKTA pure 25 see Cue Cards in the Literature list in Section *Ordering information, on page 8*.

ÄKTA start is not compatible with Superdex 30 Increase columns due to too low maximum operating pressure.

ÄKTA avant is not recommended due to non-optimal dead volumes.

Superdex 30 Increase 3.2/300 on different systems

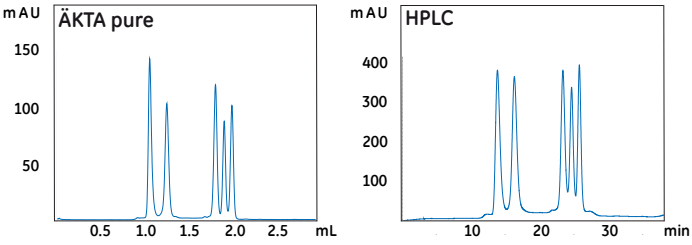


Fig 3. Comparison of protein separation on Superdex 30 Increase 3.2/300 on different systems, ÄKTA pure 25 and an HPLC system.

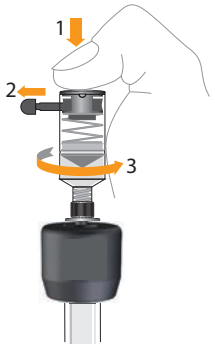
Delivery/storage

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.

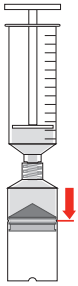
Note: *Use a flow rate for 20% ethanol according to Table 3, on page 1.*

For long term storage, 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).

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 0.15-0.3 M NaCl, pH 7.4. For hydrophobic peptides, addition of organic solvent can be beneficial. Table 4 lists some useful eluent compositions.

Table 4. Useful eluent compositions

pH	Buffer/eluent	Properties/ application examples
1.3	0.05 M HCl	Good solubility for peptides. Good UV transparency.
5.0	0.1 M ammonium acetate	Volatile.
7.2	0.05 M phosphate + 0.15 M NaCl	Physiological conditions. Very good solubility for DNA and RNA.
7.8	0.15 M ammonium hydrogen carbonate	Volatile. Should be used fresh
—	Acetonitrile 30% + TFA 0.1%	For separating hydrophobic 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	Dissociate hydrogen bonds and minimize secondary structure.
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.
Organic solvent. E.g., 30% acetonitrile	Reduces hydrophobic interaction.

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 1*.

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.

- Trifluoroacetic acid, up to 0.2%
- Isopropanol, up to 5%
- Dithiothreitol (DTT), up to 5 mM
- Acetonitrile, up to 70%
- Formic acid, up to 1%
- All commonly used aqueous buffers, pH 3 to 12
- Urea, up to 8 M
- Guanidine hydrochloride, up to 6 M
- Ionic and non-ionic detergents, e.g., 1% SDS
- Methanol, up to 70%

Short term use

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

- Sodium hydroxide, up to 1 M
- Ethanol, up to 70%
- Isopropanol, up to 30%
- Hydrochloric acid, up to 0.1 M
- Trifluoroacetic acid, up to 1%
- Dimethyl sulfoxide (DMSO), up to 10%

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 Section *Ordering information, on page 8*.

Flow rate

Action: Decrease the flow rate.

Effect: Generally improves resolution.

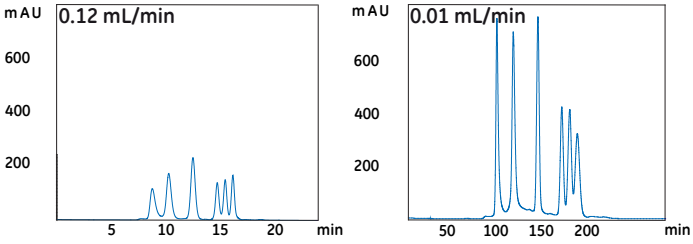


Fig 4. Comparison of peptide separation on Superdex 30 Increase 3.2/300 at different flow rates.

Sample volume

Action: Decrease the sample volume.

Effect: Improves resolution.

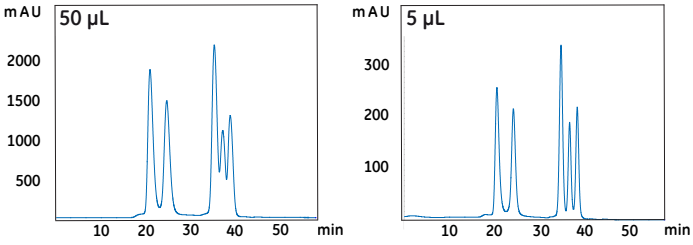


Fig 5. Comparison of peptide separation on Superdex 30 Increase 3.2/300 using different sample volumes.

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. Be aware that column bypass is not available without a column valve.

Effect: Improves resolution.

See Section *Troubleshooting, on page 7* for example of result of incorrect column valve. For further information regarding optimal configuration of ÄKTA pure 25, see Cue Cards in the Literature list in Section *Ordering information, on page 8*

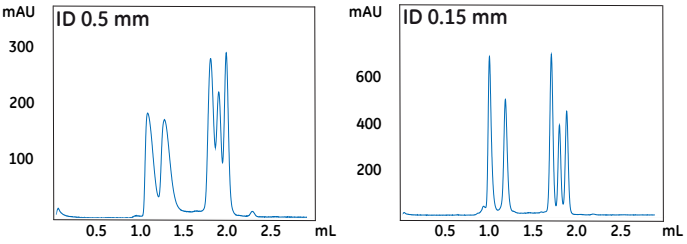


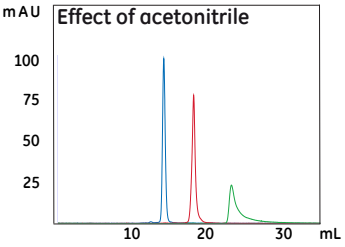
Fig 6. Comparison of peptide separation on Superdex 30 Increase 3.2/300 using different diameters of a 42 cm long capillary connected to the column.

Eluent composition

Note: *The eluent composition experiments are performed on Superdex 30 Increase 10/300 GL columns. The results show the principle and the same effects should be expected on Superdex 30 Increase 3.2/300 columns.*

Action: Add organic solvent.

Effect: Supresses hydrophobic interactions. Increases solubility of hydrophobic compounds. May change selectivity.

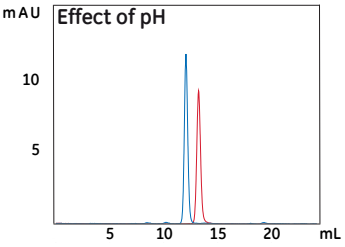


30% (blue)
15% (red)
0% (green)

Fig 7. Elution profiles of somatostatin separated in 50 mM sodium phosphate, 250 mM NaCl, pH 7.4 with different concentrations of acetonitrile.

Action: Change pH.

Effect: A pH change alters the polypeptide net charge. This can affect ionic and hydrophobic interactions.

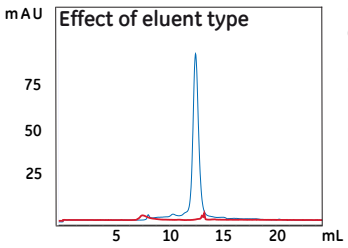


pH 2.1 (blue)
pH 10 (red)

Fig 8. Elution profiles of gastrin separated in 50 mM $NaPO_4$, 250 mM NaCl + 30% acetonitrile at different pH.

Action: Change type of eluent.

Effect: Increased ionic strength suppress ionic interaction with the resin. Note that some proteins might precipitate at low ionic strength. Buffering capacity maintain constant pH and prevents inactivation or precipitation.



Phosphate buffer with salt and organic solvent (blue)

Organic solvent with ion-pair reagent (red)

Fig 9. Elution profiles of aprotinin in 20 mM NaPO4 buffer, 280 mM NaCl, 30% acetonitrile, pH 7.4 and 30% acetonitrile + 0.1%TFA.

For more information, see the Literature list in Section *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

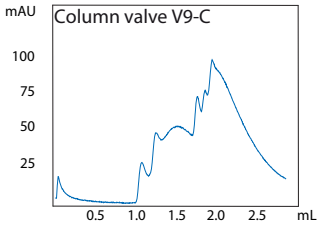
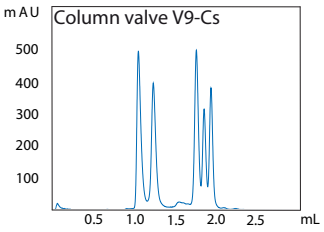
Step	Action
1	Wash the column with 1 column volume (CV) of 0.5 M sodium hydroxide, 0.5 M acetic acid, or 30%-70% acetonitrile at a flow rate of 0.02 mL/min.
2	Immediately rinse the column with 1 CV water followed by at least 2 CV of buffering eluent at a flow rate of 0.02 mL/min.

Before the next run, equilibrate the column until the UV baseline and pH are stable.

More rigorous cleaning

- Depending on the nature of the contaminants, the cleaning solutions in section *Buffers and solvent resistance, on page 5* may be used. Always rinse with at least 2 column volumes (CV) of water after any of the cleaning solutions have 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*.

Troubleshooting

Symptom	Remedy
Obtained column efficiency is not the same as in the column specification.	Result for column efficiency depends on the system and cannot be expected to be the same. Use the column efficiency for later comparisons in order to detect changes in column performance.
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 *Cleaning-in-place (CIP), on page 7*. To confirm that the high back-pressure in the system is caused by the column, disconnect one piece of equipment at atime (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.
Peak of interest is poorly resolved from other peaks.	Too high flow rate: Reduce flow rate. See *Fig. 4, on page 6*. Too high sample volume: Reduce sample volume. See *Fig. 5, on page 6*. Large extra-column volume: Minimize extra column volume in tubing. Use capillaries with reduced inner diameters. See *Fig. 6, on page 6*. Column valve with large internal volume: See examples below for an ÄKTA pure 25 system.  
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 0.05 mL/min at room temperature.
Issues with removing shipping/ storage device, stopper or connector	Carefully tighten the black end cap in both ends of the column by hand.
Other problems	See the handbook *Size Exclusion Chromatography, Principles & Methods.*

Ordering information

Product	Quantity	Product code
Superdex 30 Increase 3.2/300	1	29219758

Related products

Product	Quantity	Product code
Superdex 30 Increase 10/300 GL	1	29219757
Superdex 75 Increase 3.2/300	1	29148723
Superdex 75 Increase 5/150 GL	1	29148722
Superdex 75 Increase 10/300	1	29148721
Gel filtration LMW Calibration Kit	1	28403841
Superose™ 6 Increase 3.2/300	1	29091598
Superose 6 Increase 5/150 GL	1	29091597
Superose 6 Increase 10/300 GL	1	29091596
Superdex 200 Increase 3.2/300 GL	1	28990946
Superdex 200 Increase 5/150 GL	1	28990945
Superdex 200 Increase 10/300 GL	1	28990944

Accessories

Product	Quantity	Product code
Fingertight connector, 1/16" male	10	18111255
Tricorn™ storage/ shipping device	1	18117643

Literature

Document	Product code
Size Exclusion Chromatography, Principles & Methods	18102218
ÄKTA laboratory-scale Chromatography Systems	29010831
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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