29-0362-31 Superdex™ Peptide 3.2/300
29-0362-30 Superdex 75 3.2/300
29-0362-32 Superdex 200 3.2/300

Please read these instructions carefully before using the column.

Intended use

Superdex Peptide 3.2/300, Superdex 75 3.2/300 and Superdex 200 3.2/300 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 product in a safe way, please refer to the Safety Data Sheet.

Quick information

Superdex Peptide 3.2/300, Superdex 75 3.2/300 and Superdex 200 3.2/300 are pre-packed high performance glass columns, intended for sensitive and high resolution gel filtration of proteins, peptides, polynucleotides and other biomolecules on a micro-preparative scale.

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

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*. 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 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 0.05 ml/min.

b) Determine the column specific maximum pressure according to section *Setting column pressure limits*.

c) Equilibrate with at least 2 CV eluent at a flow rate of 0.05 ml/min.

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

Table 1. Medium and column data

Medium data	**Superdex Peptide**	**Superdex 75**	**Superdex 200**
Matrix	Dextran + cross-linked agarose	Dextran + cross-linked agarose	Dextran + cross-linked agarose
Average particle size	13 µm	13 µm	13 µm
Exclusion limit (M_r)	20 000[1]	100 000	1.3×10^6
Optimum separation range globular proteins (M_r)	100 to 7 000	3 000 to 70 000	10 000 to 600 000
pH stability range			
regular use	1 to 14	1 to 14	1 to 14
cleaning	1 to 14	3 to 12	3 to 12
Temperature			
operating	4ºC to 40ºC	4ºC to 40ºC	4ºC to 40ºC
storage	4ºC to 30ºC	4ºC to 30ºC	4ºC to 30ºC
Column data			
Bed dimensions (mm)	3.2 × 300	3.2 × 300	3.2 × 300
Approximate bed volume (ml)	2.4	2.4	2.4
Column efficiency (N/m)	≥ 30 000	≥ 30 000	≥ 30 000
Flow rate[2]			
recommended (ml/min)	0.05	0.05	0.05
maximum (ml/min)	0.15	0.1	0.1
Pressure drop over packed bed[3]	< 2.0 MPa (20 bar, 290 psi)	< 2.4 MPa (24 bar, 348 psi)	< 1.5 MPa (15 bar, 217 psi)
Column hardware pressure limit	5.0 MPa (50 bar, 725 psi)	5.0 MPa (50 bar, 725 psi)	5.0 MPa (50 bar, 725 psi)

1. Calculated by extrapolation of the linear part of the selectivity curve. In practice, molecules with a molecular weight greater than 20 000 Da will be excluded from the matrix.
2. Water at room temperature
3. Determine the limit according to section *Setting column pressure limits*

NOTICE
Make sure that the pressure limits of the column are not exceeded. 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*.

NOTICE
Always filter eluents and samples to ensure long column life. Do not apply turbid solutions (indicating sample insolubility).

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. Note that it might be significantly lower than the maximum value noted in Table 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 by one or a combination of the following factors:

- 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. 1). Some systems have additional pressure sensors located before and after the column, $\mathbf{p_{pre\text{-}cp}}$ and $\mathbf{p_{post\text{-}cp}}$.

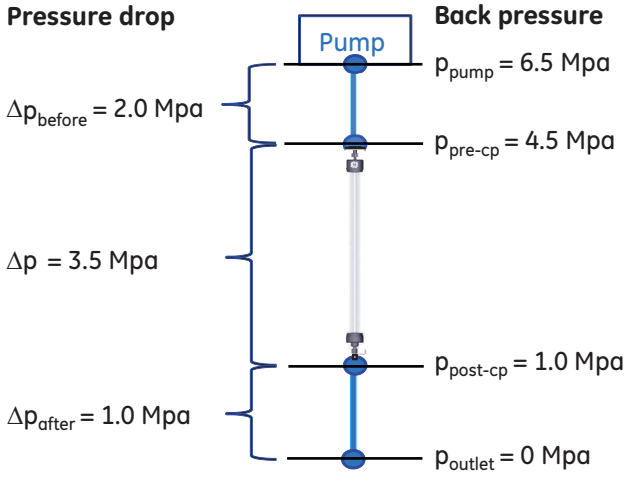

Fig 1. Example of the pressure in different parts of a system during column use. The figures are given as example values.

- $\Delta\mathbf{p_{before}}$ 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 ÄKTApurifier, 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. 1):

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

2 Equilibrate the column with room tempered water and connect the inlet 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 $\Delta\mathbf{p_{before}}$. The $\Delta\mathbf{p}$ value will be used in step 7 below.

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

4 $\Delta\mathbf{p_{before}}$ 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 $\Delta\mathbf{p_{before}}$.

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

7 Calculate $\Delta\mathbf{p} + \Delta\mathbf{p_{after}}$. If this value is lower than the column hardware pressure limit, 5.0 MPa, set the pressure limit in your method as $\Delta\mathbf{p}+\Delta\mathbf{p_{after}}+\Delta\mathbf{p_{before}}$.

You can now start your experiment!

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

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

$\Delta\mathbf{p}$ and $\mathbf{p_{pre\text{-}cp}}$ (see Fig. 1) are automatically monitored by the system. Note that the measured values includes 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, 5.0 MPa.

2 Connect the column (equilibrated with room tempered water) to the system. Start running the column at a low flow rate (e.g., 0.02 ml/min) 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 1). Note the pressure over the packed bed, $\Delta\mathbf{p}$ (DeltaC pressure), and set the value as **max delta column pressure**.

You can now start your experiment!

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

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, thus the obtained efficiency on your system might be lower compared to the specifications in Table 1.

Column efficiency test

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

$$N/m = 5.54 \times (V_R / W_h)^2 \times (1000/L)$$

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:	2 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 (Superdex Peptide), Fig. 3 (Superdex 75) and Fig. 4 (Superdex 200).

Sample:	1. Cytochrome C (M_r 12 500) 0.2 mg/ml
	2. Aprotinin (M_r 6 500) 0.2 mg/ml
	3. Gastrin I (M_r 2 126) 0.2 mg/ml
	4. Substance P (M_r 1 348) 0.2 mg/ml
	5. $(Gly)_6$ (M_r 360) 0.2 mg/ml
	6. $(Gly)_3$ (M_r 189) 0.2 mg/ml
	7. Glycine (M_r 75) 8 mg/ml
Sample volume:	5 µl
Eluent:	0.02 M phosphate buffer, 0.25 M NaCl, pH 7.2
Flow rate:	0.025 ml/min, room temperature
Detection:	214 nm

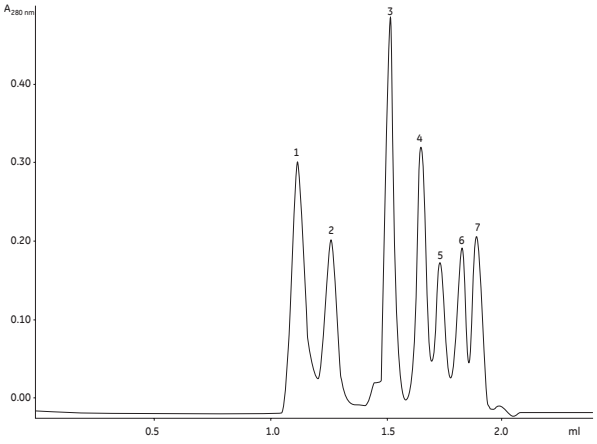

Fig 2. Typical chromatogram from a function test of Superdex Peptide 3.2/300.

Sample:	1. Transferrin (M_r 80 000) 2.0 mg/ml
	2. Ovalbumin (M_r 43 000) 2.5 mg/ml
	3. Myoglobin (horse) (M_r 17 000) 1.0 mg/ml
	4. Ribonuclease A (M_r 13 700) 5.0 mg/ml
	5. Aprotinin (M_r 6 500) 2.0 mg/ml
Sample volume:	10 µl
Eluent:	0.05 M phosphate buffer, 0.15 M NaCl, pH 7.0
Flow rate:	0.05 ml/min, room temperature
Detection:	280 nm

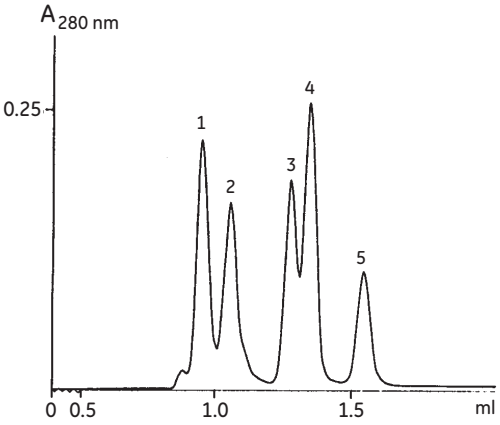

Fig 3. Typical chromatogram from a function test of Superdex 75 3.2/300.

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Sample:	1. IgG (M_r 160 000) 2.5 mg/ml
	2. Bovine serum albumin (M_r 67 000) 8.0 mg/ml
	3. β-lactoglobulin (M_r 35 000) 2.5 mg/ml
	4. Cytochrome C (M_r 12 400) 1.0 mg/ml
	5. Vitamin B_{12} (M_r 1 355) 0.1 mg/ml
	6. Cytidine (M_r 243) 0.1 mg/ml
Sample volume:	10 µl
Eluent:	0.05 M phosphate buffer, 0.15 M NaCl, pH 7.0
Flow rate:	0.04 ml/min, room temperature
Detection:	280 nm

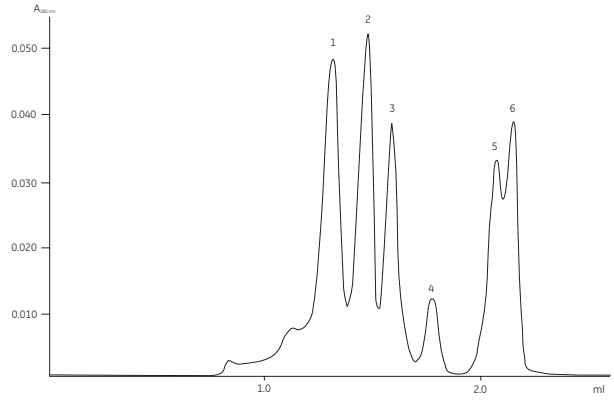

Fig 4. Typical chromatogram from a function test of Superdex 200 3.2/300.

Try these conditions first

Eluent:	0.05 M phosphate buffer, 0.15 M NaCl, pH 7.4.
Flow rate: (room temperature)	0.05 ml/min
Sample volume:	10 µl

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

Sample recommendations

Molecular weight (M_r):	Superdex Peptide:	100 to 7 000
	Superdex 75:	3 000 to 70 000
	Superdex 200:	10 000 to 600 000
Protein concentration:	Up to 50 mg/ml in sample, for higher resolution below 10 mg/ml.	
Sample volume:	< 50 µl	
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 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, or similar. Use short, narrow capillaries and avoid unnecessary components in the flow path.

Note: *Be aware of pressure limits.*

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. Some suggested buffers are listed in Table 2. To prevent non-specific ionic interactions, ionic strength equivalent to 0.05 M phosphate, 0.15 M NaCl pH 7 is recommended. This can be obtained either by adjusting the buffer concentration or adding salt, e.g. NaCl.

Table 2. Suggested buffers and eluents for various pH intervals

pH	Buffer/eluent	Properties/application examples
1.0	70% formic acid[1]	High solubility for peptides and protein fragments. Low UV-transparency at 214 nm. Volatile.
1.4	0.05 M HCl[1]	Good solubility for peptides.
5.0	0.1 M ammonium acetate	Good solubility for some enzymes, e.g. cellulases. Volatile.
<7.0	Up to 8 M urea	Good solubility for many components. Biological activity can be maintained at lower urea contents. Certain risk for carbamylation of proteins.
7.0	0.05 M phosphate, 0.15 M NaCl	Physiological conditions.
7.0	0.05 M phosphate + 6 M guanidine hydrochloride	Molecular weight determinations under denaturing 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.
11.5	0.05 M NaOH	Good solubility for some compounds.
	30% acetonitrile in suitable buffer	For separation of very hydrophobic compounds. Volatile.
	0.1% SDS, Tween or similar in suitable buffer	Good solubility for some proteins, e.g. membrane proteins.

[1] Do not expose columns to solutions with pH below 3 for more than 4 hours as this may cause limited hydrolysis of the matrix.

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.*

Superdex 75 and Superdex 200 columns can be used with aqueous solutions in the pH range 3 to 12 for normal use and 1 to 14 for cleaning. Do not expose of the column to pH below 3 for more than 4 hours since this may cause limited hydrolysis of the gel matrix. Superdex Peptide columns can be used in the pH range 1 to 14 for both normal use and cleaning, but should not be stored at pH below 3 or above 12.

Superdex Peptide, Superdex 75 and Superdex 200 are resistant to 8 M urea, 6 M guanidine-HCl, 70% formic acid and 30% acetonitrile, which are commonly used in gel filtration. The gel is also stable in alcohol/water solutions. All non-ionic or ionic detergents, such as SDS, may be used.

Avoid using oxidizing agents and unfiltered solutions. Some degradation of the polysaccharide chains may occur under oxidizing conditions.

Wetted parts of the column hardware are made of glass, PEEK and titanium, and are thus biocompatible. There is no risk of corrosion or sample contamination by metal ions.

Optimization

If your results are unsatisfactory, consider the following actions.

Action	Efect
Decrease the flow rate.	Improves resolution for high molecular weight components. The resolution for small components may be decreased.
Decrease the sample volume.	Improves resolution.
Decrease system dead volumes.	Improves resolution.
Add organic solvent.	Increases solubility of hydrophobic compounds. Changes selectivity.

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:

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

Regular cleaning:

1 Wash the column with 1 column volume (CV) of 1.0 M sodium hydroxide alternatively 0.5 M acetic acid at a flow rate of 0.02 ml/min.

2 Immediately rinse the column with 4 CV water followed by at least 4 CV 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:

- For hydrophobic proteins and lipids another cleaning method might be needed. Wash the column with 2 CV of 70% ethanol or 30% acetonitrile at a flow rate of 0.02 ml/min. Rinse with at least 4 CV of 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 37ºC. After enzymatic treatment, clean the column according to the procedure described in the section *Regular cleaning*.

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.*

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).

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 the section *More rigorous cleaning*. 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 0.05 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.
Loose end cap	Do not use the column in a system if the black end caps are loose. This is because the liquid could leak into the space between the inner column and the outer shell. Carefully tighten the black end caps by hand.

Ordering information

Product	Quantity	Code No.
Superdex Peptide 3.2/300	1	29-0362-31
Superdex 75 3.2/300	1	29-0362-30
Superdex 200 3.2/300	1	29-0362-32

Related products

Product	Quantity	Code No.
Superose 6 3.2/300	1	29-0362-26
Superose 12 3.2/300	1	29-0362-25
Gel filtration LMW Calibration Kit	1	28-4038-41
Gel filtration HMW Calibration Kit	1	28-4038-42

Accessories

Product	Quantity	Code No.
Fingertight connector, 1/16" male	10	18-1112-55
Tricorn™ storage/shipping device	1	18-1176-43

Literature

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

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29-0442-03 AB 09/2013