Superose 6 10/300 GL
Superose 12 10/300 GL

Instructions for Use

Product codes

17517201

17517301

Quick information

Superose™ 6 10/300 GL and Superose 12 10/300 GL are Tricorn™ high performance columns. The columns are pre-packed glass columns for high performance gel filtration of natural, recombinant or synthetic proteins, peptides and other biomolecules.

The column is supplied with two fingertight connectors 1/16" male for connection to ÄKTA™ design systems and two Union 1/16" male/M6 female for connection to a FPLC system.

Column data

Matrix	Composite of cross-linked agarose	
Bed dimensions	10 × 300–310 mm	
Bed volume	Approximately 24 mL	
pH stability		
regular use	3 to 12	
cleaning	1 to 14	
Temperature		
operating	4 °C to 40 °C	
storage	4 °C to 30 °C	
	Superose 6	**Superose 12**
Column efficiency, N/m	>30 000 m^{-1}	>40 000 m^{-1}
Exclusion limit, M_r, globular proteins	Approx. 4×10^7	Approx. 2×10^6
Optimal separation range (globular proteins)	5000–5×10^6	1000–3×10^5
Average particle size	13 µm	11 µm
Flow rate (water at room temperature)		
recommended	0.1–0.5 mL/min	0.5–1 mL/min
maximum	1 mL/min	1.5 mL/min
Pressure over column		
maximum	1.5 MPa, 15 bar, 218 psi	3 MPa, 30 bar, 435 psi

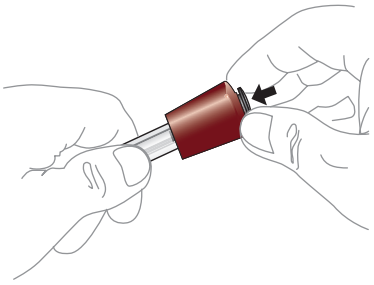


Fig 1. Illustration of how to lock the adapter. The locking ring (black) must be in the down-position to prevent uncontrolled adjustment of the column's bed-height.

First-time use

Before connecting the column to a chromatography system, ensure there is no air in the tubing and valves.

Step	Action
1	Remove the storage/shipping device and the stop plug from the column.
2	Check that the upper adapter is locked (locking ring pressed down, see figure 1).
3	Make sure that the column inlet is filled with liquid and connect it drop to- drop to the system. ***Note:*** *Superose 6 swells slightly when transformed from ethanol to water.*

To avoid local high backpressure, follow the following steps for initial equilibration:

Superose 6

Step	Action
1	12 mL distilled water at 0.2 mL/min at room temperature
2	38 mL distilled water at 0.5 mL/min at room temperature
3	50 mL eluent at 0.5 mL/min at room temperature

Note: *Ensure that the back-pressure over the column does not exceed 1.2 MPa during equilibration.*

Superose 12

Step	Action
1	50 mL distilled water at 0.2–0.5 mL/min at room temperature
2	50 mL eluent at 0.5 mL/min at room temperature

Ensure that the back-pressure over the column does not exceed 2 MPa during equilibration.

Note: *Precipitation of the sample may block the filter and cause compression of the gel. Our advice is therefore to never set the pressure limit control to more than 0.2 MPa above the actual operating pressure.*

Try these conditions first

Eluent:	50 mM phosphate buffer, 0.15 M NaCl, pH 7.0
Flow rate:	Superose 6; 0.1–0.5 mL/min, room temperature
	Superose 12; 0.5–1 mL/min, room temperature
Sample volume:	25 µL

If the viscosity of the buffers and samples are high, you may need to choose a lower flow rate to keep the pressure below the recommended limit.

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

Buffers and solvent resistance

Install an on-line filter upstream of the injection valve. Buffers and solvents with increased viscosity will affect the back-pressure and flow rate. De-gas and filter all solutions through a 0.22 µm filter.

Daily use

All commonly used aqueous buffers, pH 3–12
Urea, up to 8 M
Acetonitrile, up to 30% in aqueous buffers
Ionic and non-ionic detergents
Guanidine hydrochloride, up to 6 M
Formic acid, up to 70% (only Superose 12)

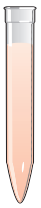
Cleaning

Acetonitrile, up to 30%
Sodium hydroxide, up to 1 M
Methanol, up to 100 %
Ethanol, up to 20% (70% for Superose 12)
Acetic acid, up to 1 M
Isopropanol, up to 24 %
Hydrochloric acid, up to 0.1 M

Avoid

Oxidizing agents
Unfiltered solutions

Sample requirements/recommendations	
Molecular weight, M_r	5 000–5 × 10^6 (Superose 6) 1 000–3 × 10^5 (Superose 12)
Protein concentration	≤ 10 mg in sample
Sample volume	25–500 µL
Preparation	Dissolve the sample in eluent, filter through a 0.22 µm filter or centrifuge at 10 000 g for 10 min.



In-depth information

Delivery/storage

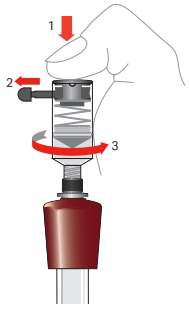
The column is delivered with a storage/shipping device that keeps the pressure in the column and thereby prevents it from drying out. The column is equilibrated with degassed 20% ethanol.

If the column is to be stored for more than 2 days after use, wash the column with 2 column volumes of distilled water and then equilibrate with at least 2 column volumes of 20% ethanol. We recommend that you connect the storage/shipping device according to "How to connect the storage/shipping device" for long term storage.

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 manufacture. The resulting uneven surface does not affect column performance or durability.

How to remove the storage/shipping device.

Step	Action
1	Push down the springloaded cap
2	Remove the locking pin
3	Release the cap and unscrew the device



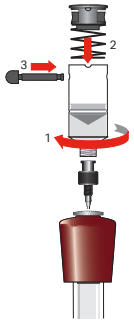
How to refill the storage/shipping device.

Step	Action
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.

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

How to connect the storage/shipping device.

Step	Action
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.
3	Secure it with the locking pin.



Choice of eluent

The eluent should be selected to ensure that the sample is fully soluble. Also, try to choose an eluent that will simplify downstream applications, if the proteins/ peptides for example are to be lyophilised a volatile eluent is necessary. As certain pH dependent interactions can occur with both acidic and basic proteins at very low salt concentrations, a recommended buffer is 50 mM sodium phosphate, 0.15 M NaCl, pH 7.

Table 1 lists some useful eluent compositions.

Table 1. Useful eluent compositions.

pH	Buffer/Eluent	Properties/application example
5.0	0.02–0.1 M ammonium acetate	Good solubility for some enzymes, e.g.cellulases. Volatile.
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.
7.0-8.0	0.02–0.1 M Tris/HCl, 1 mM EDTA	Buffer system suitable for many proteins. Very good solubility for DNA and RNA.
8.6	6 M guanidine hydrochloride in 50 mM Tris-HCl	Good UV-transparency. Suitable for large fragments of proteins that can be dialyzed to remove the guanidine.
11.5	0.05 M NaOH	Good solubility for some compounds.
Buffer additives		
	Up to 30% acetonitrile in suitable buffer Up to 8 M urea (pH <7)	For separation of very hydrophobic compounds. Volatile. Good solubility for many components. Suitable for refolding studies. Biological activity can be maintained at lower urea content. Certain risk for carbamylation of proteins.
	6 M guanidine hydrochloride	Molecular weight determinations of subunits. Suitable for refolding studies.
	0.1–2% detergent, e.g. SDS, Tween™ or similar.	Good solubility for some proteins, e.g. membrane proteins. Make sure you equilibrate completely with the detergent solution.
	0.5–10 mM Reducing agent, e.g. 2-mercaptoethanol, dithiothreitol or similar	Protect proteins against oxidation, e.g. free cysteins are not derivatized.

Optimisation

Perform a first run as described in the section "Try these conditions first". If the obtained results are unsatisfactory, consider the following:

Action	Effect
Lower flow rate	Improves resolution for high molecular weight components. The resolution for small components may be decreased.
Lower sample volume	Improves resolution
Change concentration of organic solvent	Changes selectivity
Connect two columns in series	Increased resolution due to increased bed height. Keep the total back-pressure below 2.5 MPa for Superose 6 10/300 GL and 4 MPa for Superose 12 10/300 GL.

For more information, please refer to the Handbook 18102218 which can be ordered from Cytiva or the "Method Handbook" supplied with each ÄKTA design system.

Cleaning in place (CIP)

Perform the following regular cleaning cycle after 10–20 separation cycles.

Regular cleaning:

Step	Action
1	Wash the column with 25 mL 0.5 M sodium hydroxide alternatively 0.5 M acetic acid at a flow rate of 0.5 mL/min.
2	Immediately rinse the column with 25 mL distilled water followed by at least 50 mL eluent buffer at a flow rate of 0.5 mL/min.

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

More rigorous cleaning:

Step	Action
1	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.
2	Perform a regular cleaning as described above.

Depending on the nature of the contaminants, one of the cleaning solutions on the previous page may be used. Always rinse with 2 column volumes of distilled water after any of the cleaning solutions has been used.

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" above.

If necessary, resuspend 2–3 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 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 working. Check the pressure reading after each piece is disconnected to determine the source of the back-pressure.
Air in the column	Reverse the flow and run 80–100 mL well de-gassed eluent buffer at a flow rate of 0.5 mL/min. Note that small amounts of air will normally not affect the performance of the column.

Column performance control

Check the performance of the column regularly using the following procedure:

Sample:	100 µL 0.5% acetone (5 mg/mL)
Eluent:	Distilled water
Flow rate:	0.6 mL/min, Superose 6
	0.75 mL/min, Superose 12
Detection:	280 nm

Column efficiency, expressed as 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)

As an alternative to the efficiency, test check column performance by running a function test as described in Figures 2 and 3.

Column Superose 6 10/300 GL

Column:	Superose 6 10/300 GL (Tricorn)
Sample:	1. Thyroglobulin (M_r 669 000) 5 mg/mL
	2. Ferritin (M_r 440 000) 0.4 mg/mL
	3. BSA (M_r 67 000) 8.0 mg/mL
	4. Ribonuclease A (M_r 13 700) 1.0 mg/mL
Sample volume (load):	500 µL
Eluent:	0.050 M Phosphate, 0.15 M NaCl, pH 7.0
Flow rate:	0.5 mL/min
System:	ÄKTAFPLC
Detection:	280 nm

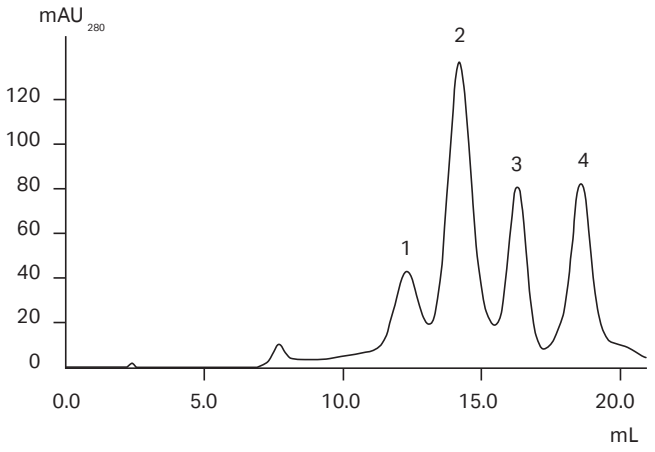


Fig 2. Typical chromatogram from a function test of Superose 6 10/300 GL.

Column Superose 12 10/300 GL

Column:	Superose 12 10/300 GL (Tricorn)
Sample:	1. IgG (M_r 150 000) 2.5 mg/mL
	2. BSA (M_r 67 000) 8.0 mg/mL
	3. β-Lactoalbumin (M_r 35 000) 2.5 mg/mL
	4. Cytochrome C (M_r 12 700) 1.0 mg/mL
	5. Vitamin B12 (M_r 1 355) 0.3 mg/mL
Sample volume (load):	500 µL
Eluent:	0.050 M Phosphate, 0.15 M NaCl, pH 7.0
Flow rate:	0.5 mL/min
System:	ÄKTAFPLC
Detection:	280 nm

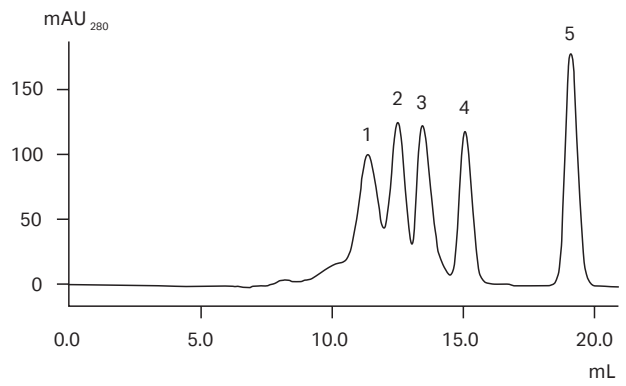


Fig 3. Typical chromatogram from a function test of Superose 12 10/300 GL.

Ordering information

Product	No. per pack	Code No.
Superose 6 10/300 GL	1	17517201
Superose 12 10/300 GL	1	17517301

Related products	No. per pack	Code No.
Superdex™ 75 10/300 GL	1	17517401
Superdex 200 10/300 GL	1	17517501
Superdex peptide 10/300 GL	1	17517601
Gel filtration LMW Calibration Kit	1	17044201
Gel filtration HMW Calibration Kit	1	17044101

Accessories	No. per pack	Code No.
TricornTricorn Filter Kit 10[1]	1	29053612
Filter tool	1	18115320
Fingertight connector, 1/16″ male	10	18111255
Union M6 female/1/16″ male	8	18111258
On-line filter (1/16″)	1	18111801
1/16″ male to luer female	2	18111251
Storage/shipping device	1	18117643
Handbook:		
Gel filtration Principles & Methods	1	18102218

[1] Do not store exposed to daylight.

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