GE Healthcare
Instructions 71-507-96 AF
High Performance Columns
17-5174-01 Superdex 75 10/300 GL and 17-5175-01 Superdex 200 10/300 GL

Quick information
Superdex™ 75 10/300 GL and Superdex 200 10/300 GL are Tricorn™ high performance columns. The columns are pre-packed glass columns for high performance gel filtration of proteins, peptides, DNA fragments ≤100 bp and other biomolecules.

The column is supplied with two fingertight connectors 1/16, male for connection to ÄKTAdesign™ systems and two union 1/16, female for connection to a FPLC™ system.

Column data
Matrix
Composite of cross-linked agarose and dextran
Bed dimensions
18 x 300-500 mm
Approximately, 1.5 ml
Average particle size
5-12 μm
Average end-to-end operating storage
+4 to +30 ºC
Storage +4 to +30 ºC
Cleaning
Avanttite, up to 50% in aqueous buffers
Acetonitrile, up to 100% methanol, up to 25% acetate, up to 70% hydrochloric acid, up to 0.2 M
Avoid
Globular proteins, M r 3 000–70 000

Sample recommendations
Molecular weight, M
3 000–70 000 (Superdex 75)
3 000–80 000 (Superdex 200)
Protein concentration, mg/ml
0.1–0.5
Preparation
20–50 μl

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 30% ethanol.

Before connecting the column to a chromatography system, ensure there is no air in or on the tubing and valves. Remove the storage/shipping device and the stop plug from the column. Check that the upper adapter is locked and ring pressed down, see figure 3. Make sure that the column inlet is filled with liquid and connect it drop-to-drop to the system.

Equilibrate the column for first-time use or after long-term storage as follows:
a) At least 50 ml distilled H2O at a flow rate of 0.5 ml/min.
b) 50 ml eluent at a flow rate of 0.5 ml/min.

Ensure that the pressure over the column does not exceed the maximum recommended pressure 0.5 MPa for Superdex 75 and 1.5 MPa for Superdex 200.

Choice of eluent
Select an eluent that ensures the sample is fully solubilized. Also try to choose an eluent that will simplify downstream applications. For example, if the proteins or peptides are to be lyophilized, a volatile eluent is necessary. As certain pH 7.0 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 7.0. Table 1 lists some useful eluent compositions.

Buffer additives
For separating very hydrophobic components. Volatile. Good solubility for some components, e.g. membrane
6 M guanidine hydrochloride
Molecular weight determinations of subunits. Good solubility for some proteins, e.g. membrane
Urea, up to 8 M
Components. Make sure you equilibrate completely with the detergent solution.

For more information, please refer to the handbook “Gel Filtration Principles & Methods”, which can be ordered from GE Healthcare, or the “Method Handbook” supplied with each ÄKTAdesign system.

Optimization
Perform as first run as described in the section “Try these conditions first” if the results obtained are unsatisfactory. Consider the following:

Action
Decrease the flow rate
Decrease the sample volume
Change the concentration of organic solvent

Effect
Improves resolution for high molecular weight components
Improves resolution for small components may be decreased
Improves resolution

Changes selectivity
Increase concentration due to increased bead weight; keep the critical bead pressure below 1.8 MPa for Superdex 75 and 1.5 MPa for Superdex 200.

Table 1. Useful eluent compositions.

<table>
<thead>
<tr>
<th>pH</th>
<th>Buffer/eluent</th>
<th>Properties/application examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.6</td>
<td>0.1 M ammonium acetate</td>
<td>Good solubility for some oligosaccharides, e.g. carbohydrates</td>
</tr>
<tr>
<td>7.2</td>
<td>0.05 M phosphate + 0.05 M NaCl</td>
<td>Physiological conditions</td>
</tr>
<tr>
<td>7.4</td>
<td>0.2 M sodium acetate</td>
<td>Suitable for some DNA and protein separations</td>
</tr>
<tr>
<td>6.0</td>
<td>0.1 M TrisCl, 1 mM EDTA</td>
<td>Very good solubility for DNA and RNA</td>
</tr>
<tr>
<td>6.4</td>
<td>6 M guanidine hydrochloride</td>
<td>Good UV transparency. Suitable if it is desired to purify proteins under denaturing conditions</td>
</tr>
<tr>
<td>6.6</td>
<td>6 M guanidine hydrochloride</td>
<td>Good solubility for some compounds</td>
</tr>
</tbody>
</table>

Try these conditions first
Eluent:
50 mM phosphate buffer, 0.15 M NaCl, pH 7.0
Flow rate:
0.5–0.75 ml/min, room temperature
Sample volume:
25 μl

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

Buffers and solvent resistance
Install an on-line filter before the injection valve. Buffers and solvents with increased viscosity will affect the bed-volume and flow rate. Do gas and filter all solutions through a 0.22 μm filter.

Dolly use
All commonly used aqueous buffers, pH 3–12
Limes, up to 8 M
Acetate, up to 50% in aqueous buffers
Ionic and non-ionic detergents
Guaridone hydrochloride, up to 6 M
Trifluoroacetic acid, up to 10% Formic acid, up to 70% Cleaning
Acetate, up to 50% Sodium hydroxide, up to 1 M Methanol, up to 25% Acetic acid, up to 1 M
Isopropanol, up to 30% Hydrochloric acid, up to 0.2 M

Avoid
Globular proteins, M r 3 000–70 000

Exclusion limit, M r
Approx. 1 × 10 5 (Superdex 75)
Approx. 1.3 × 10 6 (Superdex 200)

Flow rate (water at room temperature)
Approx. 5 ml/min
Average particle size
13 μm

Matrix Composite of cross-linked agarose and dextran
Bed volume
18 x 300-500 mm
Approximately, 1.5 ml
Average particle size
5-12 μm
Average end-to-end operating storage
+4 to +30 ºC
Storage +4 to +30 ºC
Cleaning
Acetate, up to 50% Sodium hydroxide, up to 1 M Methanol, up to 25% Acetic acid, up to 1 M Isopropanol, up to 30% Hydrochloric acid, up to 0.2 M
Avoid
Globular proteins, M r 3 000–70 000

Sample volume
25 μl
Flow rate:
0.5–0.75 ml/min, room temperature

Sample recommendations
Molecular weight, M
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0.1–0.5
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20–50 μl

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 column 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. Top off air bubbles and push the plunger to the mark on the device

How to reconnect the storage/shipping device
1. Connect a syringe or pump to the column 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. Top off air bubbles and push the plunger to the mark on the device

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.
Air in the column Run 80–100 ml well de-gassed eluent buffer at a flow rate of the source of the back-pressure.

To confirm that the high back-pressure in the system is caused by the column, disconnect one piece of equipment at a time (starting with the fraction collector) with the pumps working. Check the column, 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.

Increased back-pressure Confirm that the column is the cause (see below). If so, clean it according to the procedure described in the section “Regular cleaning”.

Symptom
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”.

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

Column performance control
Check the performance of the column using the following procedure:

Sample: BSA (Mr 67 000) 8 mg/ml
Flow rate: 0.5 ml/min.
Eluent: Buffer solution or distilled H₂O
Detection: 280 nm (mAU)

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

\[ N = \frac{5.55 \times A_w}{\lambda} \times 10^{3} \]  

where

\[ A_w \] = number of theoretical plates per meter
\[ \lambda \] = peak elution distance (m)
\[ A_w \] = peak width at half peak height (m)

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

Column Superdex 75 10/300 GL
Sample: BSA, Mr 67 000 kg/mol
Flow rate: 0.5 ml/min.
Eluent: Buffer solution or distilled H₂O
Detection: 280 nm (mAU)

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

Column Superdex 200 10/300 GL
Sample: BSA, Mr 67 000 kg/mol
Flow rate: 0.75 ml/min.
Eluent: Buffer solution or distilled H₂O
Detection: 280 nm (mAU)

Fig. 2. Typical chromatogram from a function test of Superdex 75 10/300 GL.

Fig. 3. Typical chromatogram from a function test of Superdex 200 10/300 GL.

Note: Peaks 5 and 6 are separated from each other only for difference in shape.