Try these conditions first:

- Eluent: 50 mM phosphate buffer, pH 7.0
- Flow rate: 0.6–0.7 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-off valve before the injection valve. Buffers and solvents with increased viscosity will affect the back pressure and flow rate. De-gas and filter all solvents through a 0.22 µm filter.

Daily use:

All commonly used aqueous buffers, pH 3–12

Acetaminophen, up to 30% in aqueous buffers

Ionic and non-ionic detergents

Guarindine hydrochloride, up to 6 M

Trifluoroacetic acid, up to 10 M

Formic acid, up to 70%

Cleaning:

Acetic acid, up to 20%

Sodium hydroxide, up to 1 M

Ethanol, up to 100%

Isopropanol, up to 30%

Hydrochloric acid, up to 1 M

Hydrochloric acid, up to 0.1 M

Methanol, up to 100%

Hydrochloric acid, up to 0.1 M

Sodium hydroxide, up to 1 M

Hydrochloric acid, up to 0.1 M

Avoid:

Oxidizing agents

Unfiltered solutions

In-depth information

Delivery/storage:

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

If the column is to be stored for more than 2 days after use, wash the column with 2 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.

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. As certain proteins/ peptides depend on interactions with acidic and basic proteins at very low salt concentrations, a recommended buffer is 50 mM acetic acid, 0.15 M NaCl, pH 7.0. Table 1 gives some useful eluent compositions.

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>4.4</td>
<td>0.1 M ammonium acetate</td>
<td>Great solubility for some enzymes, e.g. cellulases. Volatile.</td>
</tr>
<tr>
<td>7.2</td>
<td>0.05 M HEPES</td>
<td>Physiological conditions.</td>
</tr>
<tr>
<td>7.0</td>
<td>0.15 M ammonium hydroxide</td>
<td>Suitable for some DNA and protein separations. Volatile. Should be used with caution.</td>
</tr>
<tr>
<td>8.0</td>
<td>1 M Tris/Cl, 1 mM EDTA</td>
<td>Very good solubility for DNA and RNA. Good UV-transparency. Suitable if it is a need to purify proteins under denaturing conditions.</td>
</tr>
<tr>
<td>11.5</td>
<td>0.05 M NaOH</td>
<td>Good solubility for some compounds.</td>
</tr>
</tbody>
</table>

Buffer additives:

- 30% sucrose in 150 mM KCl
- 0.1% SDS, Tween or similar

For preparing very hydrophobic compounds.

Good solubility for many components. Biological activity can be maintained at lower urea contents. Cenetic risk for contamination of proteins.

Molecular weight determinations of substrates.

Good solubility for some proteins, e.g. membrane proteins.

Handle you equilibrate completely with the detergent solution.

Optimization:

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

<table>
<thead>
<tr>
<th>Action</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase the flow rate</td>
<td>Improves resolution for high molecular weight components. The resolution for small components may be decreased.</td>
</tr>
<tr>
<td>Decrease the sample volume</td>
<td>Improves resolution.</td>
</tr>
<tr>
<td>Change concentration of organic solvent</td>
<td>Changes selectivity.</td>
</tr>
<tr>
<td>Connect two columns in series</td>
<td>Increase resolution due to increased back-pressure. Keep the total back pressure below 1 MPa for Superdex 75, 30/300 GL and 2.5 MPa for Superdex 200 30/300 GL.</td>
</tr>
</tbody>
</table>

For more information, please refer to the handbook "Gel Filtration, Principles & Methods", which can be ordered from G-F Healthcare, or the “Method Handbook” supplied with each AKTA design system.
Cleaning in place (CIP)
Perform the following regular cleaning cycle after 10-20 separation cycles.

Regular cleaning:
1. Wash the column with 25 ml 0.1 M sodium hydroxide alternately 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.

More rigorous cleaning:
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. 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 at least 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 ºC. After enzymatic treatment, clean the column according to the procedure described in the section “Regular cleaning”. 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
Increased back-pressure over the column or pliable loss of resin
Air in the column

Remedy
Confirm that the column is the cause by bleeding. If no, check it according to the procedure described in the section “More rigorous cleaning”.

To confirm that the high back-pressure 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.

Run 80-100 ml well degassed 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 using the following procedure:

- Sample: 0.2 M NaOH ± 0.05 M NaOH ± 0.05 M NaCl ± 0.05 M NaCl
- Flow rate: 0.5 ml/min
- Detection: 280 nm

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

\[ \text{N/m} = \frac{5.54 \times (V_L/W)}{L \times W} \]

where:
- \( V_L \) = column volume from the start of sample application to the peak maximum
- \( W \) = peak width measured as the width of the recorded peak at half of the peak height
- \( L \) = bed height

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:
1. Avidin (Mw 67 000) 2 mg/ml
2. Ovuline (Mw 61 000) 2 mg/ml
3. Ribonuclease A (Mw 13 700) 1 mg/ml
4. Apotinin (Mw 4 320) 2 mg/ml
5. Vitamin B12 (Mw 1 355) 0.1 mg/ml

Sample volume: 500 µl
Flow rate: 0.5 ml/min, room temperature
Detection: 280 nm

As an alternative to the above efficiency test, check the column performance by running the function test described in Figure 3 and 4.

Column Superdex 200 10/300 GL
Sample:
1. Trypsinogen (Mw 68 000) 5 mg/ml
2. Ferritin (Mw 440 000) 0.4 mg/ml
3. BSA (Mw 66 900) 5 mg/ml
4. Lactoglobulin (Mw 15 000) 2.5 mg/ml
5. Ribonuclease A (Mw 13 700) 5 mg/ml
6. Cytochrome C (Mw 12 600) 1.5 mg/ml
7. Apotinin (Mw 4 320) 2 mg/ml
8. Vitamin B12 (Mw 1 355) 0.1 mg/ml

Sample volume: 500 µl
Flow rate: 0.5 ml/min, room temperature
Detection: 280 nm

Ordering information

Designation | No. per pack | Code No.
--- | --- | ---
Superdex 75 10/300 GL | 1 | 17-5179-01
Superdex 200 10/300 GL | 1 | 17-5179-01

Related products

Designation | No. per pack | Code No.
--- | --- | ---
Superdex Repligel 10/300 GL | 1 | 17-5174-01
Gel filtration Calibrator Kit | 1 | 28-4038-11
Gel Filtration Calibrator Kit (2) | 1 | 28-4038-11

Accessories

Designation | No. per pack | Code No.
--- | --- | ---
Triton X-100 Filter Kit | 1 | 29-0026-11
Filter tool | 1 | 18-1153-35
Finger light connector, 1/16" male | 10 | 18-1113-55
Union M6 female/1/16" male | 8 | 18-1113-58
On-line filter 1/16" | 1 | 18-1128-03
1/16" male to female | 2 | 18-1113-51
Grommets/Filtering device | 1 | 18-1176-03
Handbook | 1 | 18-1113-50
Sedimentation chromatography | 1 | 18-1022-58
Principles & Methods | 1 | 18-1022-58

* Do not store exposed to daylight.

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www.gelifesiences.com/protein-purification