**HiPrep 26/10 Desalting**

HiPrep™ 26/10 Desalting is a prepacked, ready to use column for group separation of high (M_r > 5000) from low molecular weight substances (M_r < 1000).

### Column data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrix</td>
<td>Sephadex™ G-25 Fine, cross-linked dextran</td>
</tr>
<tr>
<td>Mean particle size</td>
<td>90 μm</td>
</tr>
<tr>
<td>Bed volume</td>
<td>53 ml</td>
</tr>
<tr>
<td>Bed height</td>
<td>100 mm</td>
</tr>
<tr>
<td>i.d.</td>
<td>26 mm</td>
</tr>
<tr>
<td>Column composition</td>
<td>Polypropylene</td>
</tr>
<tr>
<td>Void volume</td>
<td>15 ml</td>
</tr>
<tr>
<td>Sample dilution</td>
<td>1:2-3 fold</td>
</tr>
<tr>
<td>Exclusion limit, proteins, peptides, M_r</td>
<td>5000</td>
</tr>
<tr>
<td>Recommended flow rate</td>
<td>9-11 ml/min (100-350 cm/h)</td>
</tr>
<tr>
<td>Maximum flow rate</td>
<td>40 ml/min (500 cm/h)</td>
</tr>
<tr>
<td>Maximum pressure over the packed bed during operation, Δp</td>
<td>0.15 MPa, 1.5 bar, 22 psi</td>
</tr>
<tr>
<td>HiPrep column hardware pressure limit</td>
<td>0.5 MPa, 5 bar, 73 psi</td>
</tr>
<tr>
<td>pH stability</td>
<td>Long term: pH 2-13, Short term: pH 2-13</td>
</tr>
<tr>
<td>Storage</td>
<td>+4 to +30 °C in 20% ethanol</td>
</tr>
</tbody>
</table>

1. Water at room temperature. Flow rate is determined by v • η < 10 ml/min where v = flow rate and η = viscosity.
2. Many chromatography systems are equipped with pressure gauges to measure the pressure at a particular point in the system, usually just after the pumps. The pressure measured here is the sum of the pre-column pressure, the pressure drop over the gel bed, and the post column pressure. It is always higher than the pressure drop over the bed alone. We recommend keeping the pressure drop over the bed below 1.5 bar. Setting the upper limit of your pressure gauge to 1.5 bar will ensure the pump shuts down before the gel is overpressured. If necessary, post-column pressure of up to 3.5 bar can be added to the limit without exceeding the column hardware limit. To determine post-column pressure, proceed as follows:

   1. Connect a piece of tubing in place of the column.
   2. Run the pump at the maximum flow you intend to use for chromatography. Use a buffer with the same viscosity as you intend to use for chromatography. Note the back pressure as total pressure.
   3. Disconnect the tubing and run at the same flow rate used in step 2. Note this back pressure as pre-column pressure.
   4. Calculate the post-column pressure as total pressure minus pre-column pressure.

To avoid breaking the column, the post-column pressure must never exceed 3.5 bar.

### Buffers and solvent resistance

- **Daily use**
  - All commonly used aqueous solutions, pH 2-13
  - Guanidine hydrochloride, up to 6 M
  - Urea, up to 8 M
  - Ethanol, up to 25%
  - Methanol, up to 25%
  - Propanol, up to 25%
  - Ionic and non-ionic detergents

- **Cleaning**
  - Sodium hydroxide, up to 0.2 M
  - Hydrochloric acid, up to 0.1 M
  - Acetic acid, up to 1.0 M

- **Avoid**
  - Solutions < pH 2
  - Solutions > pH 13
  - Oxidizing agents

### Sample recommendations

- **Sample concentration**
  - < 70 mg/ml, proteins
  - < 5 mg/ml, dextrans

- **Sample volume**
  - ≤ 15 ml

- **Preparation**
  - 0.45 μm filter or 10 000 x g for 10 min

*Note: Larger sample volumes can be applied but will have a negative influence on resolution/desalting. To increase sample volumes connect several HiPrep 26/10 Desalting columns in series. 2 x HiPrep 26/10 Desalting columns give a sample volume of 30 ml. Up to 4 x HiPrep 26/10 Desalting columns in series have been tested using 60 ml sample volume, resulting in very good results.*

### First time use

Ensure an appropriate pressure limit has been set.

Equilibrate the column for first time use or after long-term storage by running:

- At least 265 ml (5 CV) buffer at 15 ml/min.

* 1 CV = 1 column volume = 53 ml

HiPrep 26/10 Desalting can be used directly on ÄKTAdesign™ systems without the need for any extra connectors.

### Equilibration before a new run

Equilibrate with at least 265 ml (5 CV) buffer at 20 ml/min. Extended equilibration may be needed with buffers containing detergents. Equilibration is not necessary between runs with the same buffer.

Please read the back of this instruction for information on optimizing a separation.

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**HiPrep 26/10 Desalting**

**Instructions 28-4026-52 AB**

**Connectors, 2 × Union M6 female/1/16" male**

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**HiPrep 26/10 Desalting**

**Buffers and solvent resistance**

To increase column lifetime, filter all solutions through a 0.45 μm filter.

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**Delivery/storage**

The column is delivered equilibrated with 20% ethanol. If the column is to be stored for more than two days after use, clean the column according to the procedure described under “Cleaning-in-Place (CIP)” then equilibrate with at least 265 ml (5 CV) of 20% ethanol at a flow rate of 15 ml/min.

DO NOT OPEN THE COLUMN!
Choice of buffer
The buffer should be selected to ensure that the sample is fully soluble. Also, it should ideally be chosen to simplify a later stage, e.g. lyophilization or another purification step. For substances carrying charged groups an buffer containing a salt is recommended. A salt concentration of at least 0.15 M is recommended to prevent possible ionic interactions with the matrix. Sodium chloride is often used for this purpose. Suggested volatile buffers are listed in Table 1.

Table 1. Volatile buffer systems.
<table>
<thead>
<tr>
<th>pH</th>
<th>Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>3–5</td>
<td>Trimethylamine/formic acid</td>
</tr>
<tr>
<td>4–6</td>
<td>Trimethylamine/acetic acid</td>
</tr>
<tr>
<td>6.5–8</td>
<td>Trimethylamine/HCl</td>
</tr>
<tr>
<td>8.5–10</td>
<td>Ammonium carbonate/ammonia</td>
</tr>
</tbody>
</table>

Optimization
Perform your first run according to “Try these conditions first”. If the obtained results are unsatisfactory, consider the following:

Action | Effect
---|---
Decrease sample volume | Improved resolution, but greater dilution of recovered material
Decrease flow rate | Improved resolution
Decrease sample concentration | Improved resolution if close to 70 mg/ml
Connect two or more columns in series | Maintains resolution with a larger sample volume

For more information, please refer to the handbook "Gel Filtration. Principles and Methods" from GE Healthcare.

Cleaning-in-Place (CIP)
Regular cleaning
The frequency of cleaning will depend on the nature of the sample material and should be worked out on a case-by-case basis. A general cleaning procedure is the following:
1. Reverse flow direction and wash the column with 106 ml (2 CV) 0.2 M sodium hydroxide or a solution of a non ionic detergent at a flow rate of 10 ml/min. Ensure that the pressure drop does not exceed 0.15 MPa (1.5 bar, 22 psi).
2. Wash the column with 265 ml (5 CV) of distilled water at a flow rate of 15 ml/min.
3. Before the next run, equilibrate the column with at least 265 ml (5 CV) buffer until the UV base line and pH are stable.

If you suspect that the column is still contaminated, refer to the ‘More rigorous cleaning’ section.

More rigorous cleaning
To remove precipitated proteins and peptides, fill the column with 1 mg pepsin/ml in 0.1 M acetic acid, 0.5 M NaCl and leave it at room temperature overnight or 1 hour at +37 °C. After enzymatic treatment, repeat steps 1–3 in the section, “Regular cleaning”. DO NOT OPEN THE COLUMN!

Trouble shooting
<table>
<thead>
<tr>
<th>Symptom</th>
<th>Remedy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased back pressure over column and/or loss of resolution</td>
<td>Follow the procedure described in the section “Cleaning-in-Place CIP”. If back pressure is not decreased reverse the flow direction again and follow the more rigorous cleaning instruction.</td>
</tr>
<tr>
<td>Air in the column</td>
<td>Reverse the flow direction and pump 200 ml well-de-gassed buffer at a flow rate of 15 ml/min through the column. The quality of the packed bed will not normally be affected.</td>
</tr>
</tbody>
</table>

Column performance control
We recommend checking the column performance at regular intervals. The performance of the column can be checked as described in figure 1.

Table 1. Typical chromatogram from a function test of HiPrep 26/10 Desalting.

<table>
<thead>
<tr>
<th>Substance</th>
<th>pH Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>8.5–10</td>
</tr>
<tr>
<td>NHS</td>
<td>6.8–8.8</td>
</tr>
</tbody>
</table>

Figure 1. Typical chromatogram from a function test of HiPrep 26/10 Desalting.

Ordering information

<table>
<thead>
<tr>
<th>Product</th>
<th>No. per pack</th>
<th>Code No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiPrep 26/10 Desalting</td>
<td>1 × 13 ml</td>
<td>17-5087-01</td>
</tr>
<tr>
<td>HiTrap 26/10 Desalting</td>
<td>4 × 13 ml</td>
<td>17-5087-02</td>
</tr>
<tr>
<td>HiTrap™ Desalting</td>
<td>5 × 5 ml</td>
<td>17-3486-01</td>
</tr>
<tr>
<td>HiTrap™ Desalting</td>
<td>100 × 5 ml*</td>
<td>11-0003-29</td>
</tr>
</tbody>
</table>

* Special pack size delivered on specific customer order.

Accessories
To connect columns with 1/16” connectors to FPLC™ System:
- Union M6 female/1/16” male* 5 18-3858-01
- HiTrap/Hiprep 1/16” male connector to AKTAdesign 8 28-4010-81

Literature
- Handbook, Gel Filtration, Principles and Methods
- Gel Filtration, Chromatography 1 18-1124-19
- Media and Column Guide 1 18-1022-18

Further information
Check www.gehealthcare.com/protein-purification for more information.