**Introduction**

**HiPrep 16/60 or 26/60**

**Sephacryl S-100 High Resolution**

**HiPrep 16/60 or 26/60**

**Sephacryl S-200 High Resolution**

**HiPrep 16/60 or 26/60**

**Sephacryl S-300 High Resolution**

**HiPrep 16/60 or 26/60**

**Sephacryl S-400 High Resolution**

**HiPrep 16/60 or 26/60**

**Sephacryl S-500 High Resolution**

**Sephacryl High Resolution** is a cross-linked copolymer of allyl dextran and N,N-methylenebisacrylamide. This cross-linking gives the matrix high rigidity and chemical stability.

**Sleep selectivity curves** give excellent resolution power for peptides and proteins in the molecular weight range, M<sub>r</sub> 1,000–20,000 (Sephacryl S-100), M<sub>r</sub> 10,000–150,000 (Sephacryl S-200), M<sub>r</sub> 100,000–1,500,000 (Sephacryl S-300), M<sub>r</sub> 1,000,000–30,000,000 (Sephacryl S-400), and M<sub>r</sub> 3,000,000–200,000,000 (Sephacryl S-500).

See Figures 1 and 2, and “Column data” for column characteristics.

**Buffers and solvent resistance**

- **De-gas and filter all solutions through 0.22 μm filter to increase column lifetime.**
- **Buffers and solvents with increased viscosity will affect the backpressure and flow rate.**

**Sample recommendations**

- **Recommended sample load:** 0.5%–4% of the column volume (0.6–4.8 ml for 16/60, or 1.6–12.8 ml for 26/60)

**Notes:**

- **Daily use**
  - All commonly used aqueous buffers, pH 3–11
- **Cleaning**
  - Acetonitrile, up to 30%
  - Sodium hydroxide, up to 0.5 M
  - Ethanol, up to 24%
  - Acetic acid, up to 1 M
  - Isopropyl alcohol, up to 30%
  - Guanidine hydrochloride, up to 6 M
  - Urea, up to 6 M

- **Avoid**
  - Unfiltered solutions

**First time use**

These HiPrep columns can be used directly on ÄKTA™ design systems without the need for any extra connectors.

**Connecting the column**

1. Before connecting the column to a chromatography system, start the pump to remove air or from the system, particularly in tubing and valves.
2. Stop the pump.
3. Mount the column vertically, remove the bottom stop plug, and connect the inlet tubing to the system. "Stop to drop.”
4. Remove the transport device and connect the column outlet tubing to, for example, a monitor cell. Save the transport device for use when storing the column. The column is now ready for use.

**Equilibration of the column**

Ensure an appropriate pressure limit has been set. Equilibrate the column for first-time use, or after long-term storage as follows:

- **One-half column volume of distilled water at a flow rate of 15 cm/h (0.5 ml/min for 16/60, or 3 ml/min for 26/60).**
- **Two-column volumes of buffer, e.g. 0.05 M-maleic acid, 0.15 M NaCl, pH 7.2**
- **30 column volumes of 16/60 or 2.6 ml/min for 26/60.**

**Note:** Recommended flow rates are valid for H<sub>2</sub>O at 25º C. Try these conditions first

**Buffers and solvent resistance**

- **Flow rate**
  - 15 cm/h (0.5 ml/min) for 16/60, or 3 ml/min for 26/60.
- **Sample volume**
  - 1% of the column volume (0.6–4.8 ml for 16/60, or 1.6–12.8 ml for 26/60)
- **Column volume**
  - 120 ml (16/60)
  - 320 ml (26/60)

**Connecting the column**

- **Recommended flow rate**
  - 15 cm/h at room temperature
  - 30 cm/h at room temperature

**Maximum flow rate**

- 30 cm/h at room temperature
  - 60 ml/min for 16/60 or 240 ml/min for 26/60

**Maximum pressure over the packed bed during operation**

- 0.5 MPa, 1.5 bar, 73 psi

**HiPrep column hardware specifications**

- **Column volume**
  - 120 ml (16/60)
  - 320 ml (26/60)

**Recommended flow rates**

- (0.5 ml/min for 16/60 or 1.3 ml/min for 26/60)
- (2 ml/min for 16/60 or 8 ml/min for 26/60)

**Storage**

- **Storage**
  - 4°C to 30°C in 20% ethanol

**Optimization**

- **Perform a first run as described in the section “Try these conditions first”**
- **If the obtained results are unsatisfactory, consider the following:**
Cleaning-in-place (CIP)

Regular cleaning
Wash the column with one-half column volume of 0.2 M NaOH at a flow rate of 15 cm/h (0.5 ml/min for 16/60 or 1.3 ml/min for 26/60) to remove most proteins non-specifically bound to the medium. After cleaning, immediately equilibrate the column with at least two column volumes of buffer. Further equilibration is necessary if your buffer contains detergent. Wait until the UV baseline has stabilized before applying next sample.

More rigorous cleaning
Wash the column at a flow rate of 15 cm/h (0.5 ml/min for 16/60 or 1.3 ml/min for 26/60) at room temperature with the following solutions:
1. One-quarter of a column volume (0.5 ml) hot removal of hydrogenic proteins or lipoproteins, followed by four column volumes of distilled water.
2. One-half column volume 50% (v/v) isopropanol (removal of lipids and very hydrophobic proteins), followed by four column volumes of distilled water.
3. To remove precipitated proteins, digest the protein with one column volume peptic acid (0.5 M NaHCO3) overnight at room temperature. Wash with one-half column volume (0.2 ml) of 0.5 M NaCl for 16/60 or 1.3 ml/min for 26/60) to remove trace amounts of enzyme remaining in the system. Followed by four column volumes distilled water.

If a new purification is to be run, equilibrate the column after cleaning with at least five column volumes of buffer.

Note:
HiPrep columns cannot be opened or refilled.

Troubleshooting

- Increased background over the column
  Clean the column according to the section “Cleaning-in-place CIP”.
- Loss of resolution and/or decreased sample recovery
  Reverse the flow direction, and pump five column volumes of well-de-gassed water through the column at a flow rate of 30 cm/h (1 ml/min for 16/60 or 2.6 ml/min for 26/60).
- Air in the column
  Air bubbles in the column may cause a decrease in resolution. Remove air by de-gassing water before use.

Column efficiency test

We recommend checking the column performance at regular intervals. Figure 3 describes how to check the performance of HiPrep 16/60 and HiPrep 26/60 Sephacryl columns.

Column efficiency is calculated using the equation:

\[ R = \frac{V_t}{V_r} \]

where,
- \( V_t \): peak retention (elution) volume
- \( V_r \): peak width at half peak height
- \( L \): bed height (mm)

\( W_h \) and \( W_r \) in some units

### Conditions for Figures 4-6-c

**Columns**
- d) HiPrep 16/60 Sephacryl S-100 HR
- e) HiPrep 16/60 Sephacryl S-200 HR
- f) HiPrep 16/60 Sephacryl S-300 HR

**Sample**
- 500 µl of a mixture containing: IgG, 160 000, BSA-M, 67 000, B- lactoglobulin, 35 000, cytochrome C (M, 12 400) and cytidine, M = 240
- Buffer: 0.05 M sodium phosphate, 0.15 M NaCl, pH 7.0
- Flow rate: 0.8 ml/min (24 cm/h)

**Examples of column performance**
Below are examples of performance for the different HiPrep Sephacryl columns, using standard proteins or dextrans, see Figures 4-6-e.

### Conditions for Figures 4-6-e

**Columns**
- d) HiPrep 16/60 Sephacryl S-100 HR
- e) HiPrep 16/60 Sephacryl S-200 HR
- f) HiPrep 16/60 Sephacryl S-300 HR

**Sample**
- 1.2 ml of a mixture containing three dextrans: d) M > 20 000, e) M = 700 000, and f) M = 1 200 000
- Buffer: 0.25 M NaCl
- Flow rate: 0.5 ml/min (13 cm/h)

**Examples of column performance**
Below are examples of performance for the different HiPrep Sephacryl columns, using standard proteins or dextrans, see Figures 4-6-e.

### Intended use
The HiPrep Sephacryl columns are intended for research use only, and shall not be used in any clinical or in vitro procedures for diagnostic purposes.

### Ordering information

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### Accessories

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* 5 unions in the package are included in a new packaging.

### Related literature

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### Further information

For more information refer to related literature below or visit: [www.gelifesciences.com/protein-purification](http://www.gelifesciences.com/protein-purification)