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# HiTrap G-25 Desalting column, 5 mL

## Catalog Number: HP009, HP010

**Table 1. Package and Storage**

| Cat No. | Material | Unit Size | Storage | Stability |
|---|---|---|---|---|
| **HP009** | HiTrap G-25 Desalting column | 1 × 5 mL | 2-30 °C<br>20% ethanol | The product is stable for at least 24 months when stored as directed. |
| **HP010** | HiTrap G-25 Desalting column | 5 × 5 mL | | |

## Product Description

HiTrap G-25 Desalting are ready-to-use 5 mL columns prepacked with cytiva Sephadex G-25 Superfine resin for fast and convenient desalting and buffer exchange.

The exclusion limit is approximately $M_r$ 5000. This ensures group separation of proteins/peptides larger than $M_r$ 5000 from smaller molecules.

The special design of the column, together with the well-known resin Sephadex G-25 Superfine, provides fast and reproducible separations in a convenient format. The column can be operated with a syringe, peristaltic pump or liquid chromatography system such as ÄKTA™.

### HiTrap Column Characteristics

The columns are made of biocompatible polypropylene that does not interact with biomolecules.

The columns are delivered with a stopper at the inlet and a snap-off end at the outlet. Table 1 lists the characteristics of HiTrap columns.


**Table 1. Characteristics of HiTrap columns.**

| | |
|---|---|
| Column volume (CV) | 5 mL |
| Column dimensions | 1.6 × 2.5 cm |
| Column pressure limit | 5 bar (0.5 MPa) |

### Resin Properties

The HiTrap Desalting column is packed with the well-known size exclusion medium Sephadex G-25 Superfine. The medium is based on cross-linked dextran beads which allow excellent resolution and high flow rates. The fractionation range for globular proteins is between Mr 1000 and 5000, with an exclusion limit of approximately Mr 5000. This ensures group separations of proteins/peptides larger than Mr 5000 from molecules with a molecular weight less than Mr 1000.

HiTrap Desalting can be used with aqueous solutions in the pH range 2 to 13. It is stable to all commonly used buffers, solutions of urea (8 M), guanidine hydrochloride (6 M), and all non-ionic and ionic detergents. Lower alcohols (methanol, ethanol, propanol) may be used in the buffer or the sample, but we recommend that the concentration be kept below 25 v/v%. Prolonged exposure (hours) to pH values below 2 or above 13, or to oxidizing agents should be avoided.

The recommended range of sample volumes is 0.1 to1.5 ml when complete removal of low molecular weight components is desired. The separation is not affected by the flow rate, in the range 1 to 10 ml/min. The maximum recommended flow rate is 15 ml/min.

Characteristics of the HiTrap Desalting column are summarized in Table 2.

**Table 2. HiTrap G-25 Desalting characteristics**

| | |
|---|---|
| Matrix | Sephadex G-25 Superfine, cross-linked dextran |
| Particle size | 20 to 50 µm |
| Separation mechanism | According to size |
| Void volume | 1.5 mL |
| Sample volume | 0.1 to 1.5 mL |
| Exclusion limit | Mr 5 000 |
| Recommended flow rate | 1 to 10 mL/min |
| Chemical stability | All commonly used buffers |
| pH stability | 2 to 13 |
| Storage | 20% ethanol, 2°C to 30°C |

## Operation

### Buffer preparation

For substances carrying charged groups an eluent containing a buffer salt is recommended. A salt concentration of at least 25 mM is recommended to prevent possible ionic interactions with the matrix. Sodium chloride is often used for this purpose. At salt concentrations above 1.0 M, hydrophobic substances may be retarded or bind to the matrix. At even higher salt concentrations (>1.5 M $(NH_4)_2SO_4$), the column packing shrinks.

### Sample preparation

The sample concentration does not influence the separation as long as the viscosity does not differ more than a factor of 1.5 from that of the buffer used. This corresponds to a maximum concentration of 70 mg/ml for proteins or 5 mg/ml for high molecular weight polymers such as dextran, when normal aqueous buffers are used. The sample should be fully solubilized. Centrifuge or filter (0.45 µm filter) immediately before loading to remove particulate material if necessary.

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## Method

1. Fill the syringe or pump tubing with buffer. Remove the stopper. To avoid introducing air into the column, connect the column “drop to drop” to either the syringe (via the connector) or to the pump tubing.
2. Remove the snap-off end at the column outlet.
3. Equilibrate the column with 25 ml buffer at 5 ml/min to completely remove the ethanol.
4. Apply up to 1.5 ml of sample. Monitor the effluent from the column with a UV monitor and/or a conductivity monitor. Keep the flow rate in the range 1 to 10 ml/min. Collect fractions.
5. Elute the column with approximately 10 ml buffer before applying the next sample. Collect fractions.

## Storage

Before storage, we recommend to wash the column with 5 column volumes of 20% ethanol to prevent microbial growth. Seal the column with the supplied stoppers. Store the HiTrap G-25 Desalting column at 2°C to 30°C.