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HiTrap DEAE Sephadex A-25 column, 5 mL

Catalog Number: HP011, HP012

Table 1. Package and Storage

Cat No.	Material	Unit Size	Storage	Stability
HP011	HiTrap DEAE Sephadex A-25 column	1 × 5 mL	2-30°C 20% ethanol	The product is stable for at least 24 months when stored as directed.
HP012	HiTrap DEAE Sephadex A-25 column	5 × 5 mL		

Product Description

HiTrap DEAE Sephadex A-25 are ready-to-use 5 mL columns prepacked with cytiva DEAE Sephadex A-25 resin for small-scale purification using weak anion exchange resin.

DEAE Sephadex A-25 is weak anion exchanger. The ion exchange group is diethylaminoethyl which remains charged and maintains consistently high capacity below pH 9.

The special design of the column, together with the well-established chromatography resin DEAE Sephadex A-25, provides fast, reproducible, and easy 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

DEAE Sephadex A-25 is a weak anion exchanger based on the well documented and well proven Sephadex base matrix.

DEAE Sephadex ion exchanger is produced by introducing diethylaminoethyl functional group onto the cross-linked dextran matrix. This group is attached to glucose units in the matrix by stable ether linkages.

Characteristics of the HiTrap DEAE Sephadex A-25 are summarized in Table 2.

Table 2. HiTrap DEAE Sephadex A-25 characteristics

Matrix	Cross-linked dextran, spherical
Particle size	40 to 100 µm
Ion exchange type	Weak anion
Ionic capacity	3.0-4.0 mmol/g dry resin
pH ligand fully charged	Below 9
pH stability	2 to 13
Recommended flow rate	1 to 10 mL/min
Chemical stability	All commonly used buffers
Autoclavability	30 min at 121°C in 0.1 M NaCl
Storage	20% ethanol, 2°C to 30°C

Operation

Selection of buffer pH and ionic strength

Buffer pH and ionic strength are critical for the binding and elution of material (both target substances and contaminants) in ion exchange chromatography.

Start buffer

The concentration of buffer required to give effective pH control varies with the buffer system. In most cases a concentration of at least 10 mM is required to make sure that there is adequate buffering capacity. The ionic strength of the buffer must be kept low (< 5 mS/cm) so as not to interfere with sample binding. Salts also play a role in stabilizing protein structures in solution, and it is important the ionic strength are not so low that protein denaturation or precipitation occurs.

The buffering ion must carry the same charge as the ion exchange group and should have a pKa within 0.5 pH units of the pH used in the separation.

Starting pH

At least 1 pH unit above the pI of substance to be bound.

Buffer preparation

Water and chemicals used for buffer preparation must be of high purity. It is recommended to filter the buffers by passing them through a 0.45 µm filter before use.

Sample preparation

The sample must be adjusted to the composition of the start buffer by buffer exchange using Desalting columns. The sample must be filtered through a 0.45 µm filter or centrifuged immediately before it is applied to the column.

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Purification

1. Fill the syringe or pump tubing with start 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 start buffer at 5 ml/min to completely remove the ethanol.
4. Wash with 5 column volumes of elution buffer (start buffer with 1 M NaCl).
5. Finally equilibrate with 5 to 10 column volumes of start buffer.
6. Apply the sample at 5 mL/min using a syringe fitted to the luer connector or by pumping it onto the column.
7. Wash with at least 5 column volumes of start buffer or until no material appears in the eluate.
8. Elute with 5 to 10 column volumes of elution buffer.
9. The purified eluted fractions can be desalted using desalting column if necessary.
10. After completed elution, regenerate the column by washing with 5 column volumes of regeneration buffer (start buffer with 1 M NaCl) followed by 5 to 10 columns volumes of start buffer. The column is now ready for a new sample.

Storage

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