Instructions

High Capacity IEX / Mixed-Mode Selection Kit

Five different ion-exchange and mixed-mode chromatography resins are prepacked to 1 ml ready to use columns for screening purpose. The column design provides fast, simple and easy separations in a convenient format.

The columns can be operated with a syringe, peristaltic pump or common liquid chromatography system such as ÄKTA™ when suitable tubing adaptors are used.

Please read these instructions carefully before using the columns.

Intended use

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

Safety

For use and handling of the product in a safe way. The resins contain 20% ethanol to prevent microorganisms from growing. Proper PPE (e.g. gloves and goggles) must be used to handle the columns.

Product description

Column characteristics

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

The columns are delivered in sealed plastic bags with identical stop plug to each end.

Table 1. Characteristics of column hardware

Column volume (CV)	1 ml
Column dimensions	6.2 × 33 mm
Column hardware pressure limit	5 bar (0.5 MPa)

Note: *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium, sample/liquid viscosity and the column tubing used.*

Properties of High Capacity IEX / Mixed-Mode resins

Table 2. IEX / MM resins included in the kit

Resin name	Brief description
Q SepFast 6HF Plus	Strong anion-exchange resin made of highly cross-linked 6% agarose and dextran; it offers much increased binding capacity and binding kinetics than Q SepFast 6HF
DEAE SepFast 6HF Plus	Weak anion-exchange resin made of highly cross-linked 6% agarose and dextran; it offers much increased binding capacity and binding kinetics than DEAE SepFast 6HF
SP SepFast 6HF Plus	Strong cation-exchange resin made of highly cross-linked 6% agarose and dextran; it offers much increased binding capacity and binding kinetics than SP SepFast 6HF
SepFast MMWC-1	Mixed-mode ligand having weak cation-exchange (carboxyl) and hydrophobic (phenyl) moieties. The binding capacity could be maintained over a broad range of salt concentrations.
MabPolish Type I	A special anion mixed-mode resin tailored for the removal of host cell proteins from antibody cell culture stream.

The general properties of the resins are summarized in Table 3.

Table 3. Key properties of SepFast IEX / MM resins

Bead structure	Highly cross-linked agarose (with dextran in the Plus range)
Mean particle size	50 - 150 µm
Rec. flow rates	0.2 to 1 ml/min
Chemical stability	All commonly used buffers
pH stability	
Long term	3 to 12
Short term	2 to 13
Storage	2ºC to 8ºC in 20% ethanol

Operation

The column can be operated with a syringe, peristaltic pump or a chromatography system. Suitable tubing adaptors are required (contact Us for further information).

We recommend scouting the parameters among loading capacity, flow velocity, binding pH, binding ionic strength, elution speed and gradient etc. Due to the fast binding kinetics of Q and SP SepFast 6HF Plus resins, the binding step could be done in a faster flow velocity than that in the elution step. We recommend to pay special attention to optimize elution conditions to achieve the best separation power.

Strong ion exchange media maintain their charges (and thus their function) over a wide pH range whereas with weak ion exchange media the degree of dissociation and thus ion exchange capacity varies with pH. Therefore, it is more critical to optimize the pH if weak ion exchange media is used.

At pH 4 to 5 and salt concentration up to 0.15 M, MabPolish Type I can remove a broad range of host cell proteins from monoclonal antibody (Mab) cell culture, with little binding to the target Mab. pH, conductivity and type of salt could be optimized according to the property of a given Mab.

In general, balancing the degree of component separation against process throughput is the major consideration when optimizing a method. Besides, for the purification of instable or shearing-force sensitive molecules, the operational condition needs be optimised to balance the throughput and the possible damage to the target molecule.

Purification

The recommended flow rate is 0.2 to 1 ml/min.

1. Fill the syringe or pump tubing with binding buffer. Follow the flow direction. Remove the top stop plug and connect the column to the syringe (with the provided connector), or pump tubing, “drop to drop” to avoid introducing air into the column.
2. Remove the stop plug at the column outlet.
3. Wash out the preservative and equilibrate the column with 10 column volumes of binding buffer.
4. Apply the sample, using a syringe fitted to the luer connector or by pumping it onto the column.
5. Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.
6. Elute with 5 to 10 column volumes of elution buffer using a continuous or step gradient.

Storage

Wash the column with at least 2 column volumes of 20% ethanol (or 20% ethanol + 0.2M sodium acetate for SP SepFast 6HF Plus) at reduced flowrates such as

0.5 ml/min. Store the columns at 2ºC to 8ºC. Regular check of column performance is recommended.

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