Instructions

HiSep MBP SepFast HighRes

1 ml and 5 ml HiSep MBP SepFast HighRes is a prepacked ready to use, column for preparative affinity chromatography. 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

HiSep 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 resin is stored in 20% denatured ethanol to prevent microorganisms from growing. Proper PPE (e.g. gloves and goggles) must be used to handle the columns.

Product description

HiSep 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 (see Figure 1). The snap-off is used as the sealing plug for the outlet of the column. The inlet has 1/16" female thread connection. The outlet has 1/16" male thread connection compatible to most Akta systems.

Table 1 lists the characteristics of HiSep columns.

Figure 1 . HiSep 1 ml and 5 ml column.

Note:*HiSep columns cannot be opened or refilled.*

Note:*Make sure that the connector is tight to prevent leakage.*

Table 1. Characteristics of HiSep columns.

Column volume (CV)	1 ml	5 ml
Column dimensions	0.7 × 2.5 cm	1.6 x 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa)	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.*

Medium properties

MBP SepFast HighRes is a chromatography medium for purifying recombinant proteins tagged with maltose binding protein (MBP). It is a scalable medium.

Affinity purification using MBP SepFast HighRes takes place under physiological conditions and mild elution is performed using maltose. This preserves the activity of the target protein. Even intact protein complexes may be purified. In addition, the high specificity of the binding means that very high purity can be achieved in just one step in combination with high binding capacity.

MBP SepFast HighRes is a robust, high resolution medium based on the well known agarose matrix. The small and evenly sized beads ensure that MBP-tagged proteins elute in narrow peaks, thus minimizing the need for further concentration steps. MBP SepFast HighRes tolerates all commonly used aqueous buffers and is easily regenerated using 0.5 M NaOH allowing the same medium to be used for repeated purifications.

The properties of the product are summarized in Table 2.

Table 2. HiSep MBP SepFast HighRes characteristics

Ligand	Dextrin
Binding capacity	Protein dependent
Bead structure	Highly cross-linked agarose
Mean particle size	25 - 50 µm
Rec. flow rates	0.2 to 1 ml/min for 1 ml column 1 to 3 ml/min for 5 ml column
Max. flow rates	Never exceed the pressure of 5 bar
Chemical stability	All commonly used buffers
pH stability	
Long term	5 to 10
Short term	5 to 10
Storage	4ºC to 30ºC in 20% ethanol

Operational conditions

The commonly used methods for MBP protein purifications can be directly adopted to this resin. The common buffers can be tris or phosphates at pH >7. Salts, EDTA or DTT can be added to adjust the binding conditions or to stabilise the target proteins. 10 mM to 50 mM maltose in the binding buffer is normally employed to elute the MBP.

The presence of reducing agents, e.g., 5 mM DTT, may decrease yield. Higher ionic strength does not decrease affinity since MBP binds to the resin primarily by hydrogen binding. Agents that interfere with hydrogen binding, such as urea and guanidine hydrochloride, are not recommended. The presence of 10% glycerol may decrease the yield and 0.1% SDS completely eliminates the binding

In general, balancing product recovery against process throughput is the major consideration when optimizing a method.

Regeneration

After each run, elute any reversibly bound material with 0.1 to 0.5 M sodium hydroxide.

Sample preparation

The sample should be adjusted to the composition of the binding buffer. This can be done by either diluting the sample with binding buffer or by buffer exchange using desalting columns.

Purification

The recommended flow rate is 0.2 to 1 ml/min or 1 to 3 ml/min for 1 ml or 5 ml column, respectively.

1. Fill the syringe or pump tubing with binding buffer. Remove the stopper 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 snap-off end at the column outlet. Keep the snap-off part as stop plug for the 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.
7. The purified fractions can be desalted.

Storage

Wash the column with 3 column volumes of 20% ethanol at reduced flowrates such as 0.2 ml/min (HiSep 1 ml column) or 1 ml/min (HiSep 5 ml column). Store the column in 20% ethanol in a cold room.

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