Instructions

HiSep Heparin SepFast HighRes

1 ml and 5 ml HiSep Heparin 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% isopropanol 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.

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

The nature of Heparin SepFast™ HighRes makes it a very versatile tool for the separation of many proteins e.g., DNA binding enzymes, growth factors, coagulation factors and steroid receptors.

The ligand is a naturally occurring sulfated glucosaminoglycan which is extracted from the native proteoglycan of porcine intestinal mucosa. Heparin consists alternating units of uronic acid and D-glucosamine, most of which are substituted with one or two sulfate groups. Heparin is covalently coupled to highly cross-linked agarose beads. The coupling method gives high capacity and high performance.

The medium is stable over the pH range 5 to 10, and tolerates all commonly used aqueous buffers.

The properties of the product are summarized in Table 2.

Table 2. HiSep Heparin SepFast HighRes characteristics

Ligand	Heparin
Degree of substitution	~6 mg heparin/ml medium
Bead structure	Highly cross-linked agarose
Mean particle size	20 -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 or 4ºC in 0.02% sodium azide

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).

Immobilized heparin has two main modes of interaction with proteins. It can operate as an affinity ligand; e.g., in its interaction with coagulation factors. Heparin has also a function as a high capacity cation exchanger due to its anionic sulphate groups.
Recommended elution conditions for both cases is to increase the ionic strength. Elution using a continuous or step gradient with NaCl, KCl or $(NH_4)_2SO_4$ up to 1.5 to 2 M is most frequently used.

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

Recommended buffers:

Binding buffer: 10 mM sodium phosphate, pH ~7

Elution buffer: 10 mM sodium phosphate, 1-2 M NaCl, pH ~7

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 5 column volumes of 20% ethanol at reduced flowrates such as 0.5 ml/min (HiSep 1 ml column) or 1.5 ml/min (HiSep 5 ml column). Store the column in 20% ethanol at 4ºC to 30ºC. Alternatively, Wash the column with 0.02% sodium azide and store at 4ºC. Regular check of the column performance is recommended.

BioToolomics Ltd
Unit 30A, Number 1 Industrial Estate
Consett
County Durham, DH8 6TJ
United Kingdom

www.biotoolomics.com