

# Instructions

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## Protein A SepFast Column

1 ml

5 ml

Protein A SepFast chromatography resin is prepacked to 1 ml and 5 ml ready to use columns for purification of antibodies. The column design provides fast, simple and easy separations in a convenient format.

The column 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 resin is stored in 20% isopropanol during shipping 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 x 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.

**Table 2. Key properties of Protein A SepFast resin**

Bead structure	Agarose bead
Mean particle size	50 - 150 µm
Binding capacity	>30 mg/ml human IgG
Chemical stability	All commonly used buffers
pH stability	
Long term	2 to 10
Short term	3 to 9
Storage	4°C in 20% ethanol (or isopropanol)

### 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 and flow velocity etc to achieve the best desalting performance.

The recommended flow rate is 0.1 to 0.5 ml/min for 1 ml column and up to 2.5 ml/min for the 5 ml column.

- 1 Fill the syringe or pump tubing with equilibration 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 snap-off end at the column outlet. Keep the snap-off part as stop plug for the outlet.
- 3 Wash out the preservative (at reduced flowrate) and equilibrate the column with at least 5 column volumes of equilibration buffer.
- 4 Apply the sample, using a syringe fitted to the luer connector or by pumping it onto the column.
- 5 Wash the column with a suitable buffer.
- 6 Elute is normally done at reduced pH (e.g. 2.8 to 3.2).

### Storage

Wash the column with 5 column volumes of 20% ethanol at reduced flowrates such as 0.2 ml/min (1 ml column) or 1 ml/min (5 ml column). Store the column in 20% ethanol at 4°C.



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