Instructions

Protein G SepFast HighRes Column

1 ml
5 ml

1 ml and 5 ml Protein G SepFast HighRes column 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

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% ethanol or 20% isopropanol 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 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)
Resin pressure limit	2 bar (0.2 MPa)	2 bar (0.2 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

Protein G SepFast HighRes is affinity resin with protein G immobilized to highly cross-linked smaller agarose-based beads. It allows to capture antibodies at reduced contact time due to short diffusion distance within the beads.

Protein G binds to the Fc region of IgG from a variety of mammalian species. Protein G SepFast HighRes may be used to isolate and purify classes, subclasses and fragments of immunoglobins from any biological fluid or cell culture medium. Protein G SepFast HighRes is extremely useful for isolation of immune complexes.

The potential applications of protein G include practically all of the current and projected applications of protein A. Protein G and protein A, however, have different IgG binding specificities, dependent on the origin of the IgG. Compared to protein A, protein G binds more strongly to polyclonal IgG, for example, from cow, sheep and horse. Furthermore, unlike protein A, protein G binds polyclonal rat IgG, human IgG_3 and mouse IgG_1.

The properties of the product are summarized in Table 2.

Table 2. Characteristics of Protein G SepFast HighRes columns

Ligand	Rec. protein G with the albumin binding domain depleted
Mean particle size	20 -50 µm
Rec. flow rates	0.2 to 0.5 ml/min for 1 ml column 1 to 2.5 ml/min for 5 ml column
Max. flow rates	Never exceed the resin bed pressure of 2 bar and column pressure of 5 bar
Chemical stability	All commonly used buffers
pH stability	
Long term	3 to 9
Short term	2 to 10
Storage	4ºC in 20% ethanol (or 20% isopropanol) or 0.02% sodium azide

Purification

Please refer the details in the manual for the loose Protein G resin.

Column operation

The recommended flow rate is 0.2 to 0.5 ml/min or 1 to 2.5 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 0.2 ml/min (HiSep 1 ml column) or 1 ml/min (HiSep 5 ml column). Store the column in 20% ethanol at 4ºC to 30ºC. 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