



HiTrap Protein A HP, 1 ml and 5 ml

Instructions for Use

HiTrap™ Protein A HP is a prepacked ready to use, column for preparative purification of monoclonal and polyclonal antibodies. The special design of the column, together with the medium provide fast, simple and easy separations in a convenient format.

The column can be operated with a syringe, peristaltic pump or liquid chromatography system such as ÄKTA™.

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Important

Please read these instructions carefully before using HiTrap columns.

Intended use

HiTrap 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, please refer to the Safety Data Sheet.

1 Product description

HiTrap 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. Table 1 lists the characteristics of HiTrap columns.



Fig 1. HiTrap, 1 ml column.



Fig 2. HiTrap, 5 ml column.

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

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

Table 1. Characteristics of HiTrap columns.

Column volume (CV)	1 ml	5 ml
Column dimensions	0.7 × 2.5 cm	1.6 × 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.*

Supplied Connector kit with HiTrap column

Connectors supplied	Usage	No. supplied
Union 1/16" male/ luer female	For connection of syringe to HiTrap column	1
Stop plug female, 1/ 16"	For sealing bottom of HiTrap column	2, 5 or 7

Medium properties

HiTrap Protein A HP 1 ml and 5 ml columns are packed with 1 ml and 5 ml of Protein A Sepharose™ High Performance, respectively.

Protein A Sepharose High Performance is designed for purification and isolation of monoclonal and polyclonal IgG from ascites, serum and cell culture supernatants (Table 3).

Protein A is produced by a selected strain of *Staphylococcus aureus* and has a molecular weight of 42 000. Protein A consists of six different regions, five of which show strong, specific binding for the Fc-part of IgG, leaving the antigen-binding sites free. Immobilized protein A can bind at least two molecules of IgG per molecule.

In general, one can purify most IgGs using protein A.

Purified protein A is coupled to highly cross-linked agarose beads by the N-hydroxysuccinimide activation method. This coupling method gives high capacity and high performance.

The binding capacity of protein A for IgG depends on the source species of the particular immunoglobulin, see Table 3 and 4. The total capacity depends also upon several other factors such as the flow rate during sample application, and the sample concentration. This medium has a binding capacity for human IgG of approximately 20 mg IgG/ml medium.

The characteristics of HiTrap Protein A HP are summarized in Table 2.

Table 2. HiTrap Protein A HP characteristics

Ligand	Protein A, $M_r \sim 42\ 000$
Degree of substitution	~ 3 mg protein A/ml medium
Binding capacity	~ 20 mg human IgG/ml medium
Mean particle size	34 μm
Max. back pressure	0.3 MPa, 3 bar
Bead structure	Highly cross-linked spherical agarose
Max. flow rates ¹	4 and 20 ml/min for 1 ml and 5 ml column respectively
Recommended flow rates	1 ml/min and 5 ml/min for 1 ml and 5 ml column respectively
Chemical stability	All commonly used buffers
pH stability ²	
long term	3 to 9
short term	2 to 9
Storage	2°C to 8°C in 20% ethanol

¹ Water at room temperature.

² The ranges given are estimates based on our knowledge and experience. Please note the following:

pH stability, long term refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.

pH stability, short term refers to the pH interval for regeneration, cleaning-in-place and sanitization procedures.

pH below 3 is sometimes required to elute strongly bound IgG species. However protein ligands may hydrolyze at very low pH.

Table 3. Relative binding strengths for protein A and protein G

Species	Subclass	Protein A binding	Protein G binding
Human	IgA	variable	–
	IgD	–	–
	IgE	–	–
	IgG ₁	++++	++++
	IgG ₂	++++	++++
	IgG ₃	–	++++
	IgG ₄	++++	++++
	IgM*	variable	–
Avian egg yolk	IgY†	–	–
Cow		++	++++
Dog		++	+
Goat		–	++
Guinea pig	IgG ₁	++++	++
	IgG ₂	++++	++
Hamster		+	++
Horse		++	++++
Koala		–	+
Llama		–	+
Monkey (rhesus)		++++	++++
Mouse	IgG ₁	+	++++
	IgG _{2a}	++++	++++
	IgG _{2b}	+++	+++
	IgG ₃	++	+++
	IgM*	variable	–
Pig		+++	+++
Rabbit	no distinction	++++	+++
Rat	IgG ₁	–	+
	IgG _{2a}	–	++++
	IgG _{2b}	–	++
	IgG ₃	+	++
Sheep		+/-	++

* Purify using HiTrap IgM Purification HP columns.

† Purify using HiTrap IgY Purification HP columns.

++++ = strong binding

++ = medium binding

– = weak or no binding

Table 4. Affinity of protein A for selected classes of monoclonal antibodies. This table is compiled from a variety of sources. Comparisons should be understood to be approximate since they are derived from runs conducted under a variety of conditions.

Antibody	Affinity	Binding pH	Elution pH
Human			
IgG ₁	very high	6.0–7.0	3.5–4.5
IgG ₂	very high	6.0–7.0	3.5–4.5
IgG ₃	low-none	8.0–9.0	≤ 7.0
IgG ₄	low-high	7.0–8.0	3.0–6.0
Mouse			
IgG ₁	low	8.0–9.0	4.5–6.0
IgG _{2a}	moderate	7.0–8.0	3.5–5.5
IgG _{2b}	high	~7.0	3.0–4.0
IgG ₃	low-high	~7.0	3.5–5.5

2 Operation

Protein A binds IgG over a wide pH range, and thus permits the use of a wide variety of buffers, depending on the applications. Elution is often achieved by a decrease in pH. Different subclasses of IgG elute at different pH values depending on the species from which they originate.

As a safety measure to preserve the activity of acid labile IgG when using very acidic elution conditions, we recommend you to add 60 to 200 µl of 1 M Tris-HCl, pH 9.0, to those tubes destined to collect fractions containing IgG, so that the final pH of the sample will be approximately neutral.

The column can be operated with a syringe, peristaltic pump or a chromatography system.

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	20 mM sodium phosphate, pH
Elution	0.1 M citric acid, pH 3–6

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 HiTrap Desalting, PD-10 Desalting or HiPrep™ 26/10 Desalting columns, see Table 5. The sample should be filtered through a 0.45 µm filter or centrifuged immediately before it is applied to the column. (This is especially important to prevent clogging of the column when loading large volumes of serum or plasma).

3 Purification

- 1 Prepare collection tubes by adding 60 to 200 μ l of 1 M Tris-HCl, pH 9.0 per ml of fraction to be collected.
- 2 Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided luer connector), or pump tubing, "drop to drop" to avoid introducing air into the column.
- 3 Remove the snap-off end at the column outlet.
- 4 Wash the column with 10 column volumes of binding buffer at 1 ml/min or 5 ml/min for 1 ml and 5 ml column respectively.
- 5 Apply the sample, using a syringe fitted to the luer connector or by pumping it onto the column.
- 6 Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.
- 7 Elute with 2 to 5 column volumes of elution buffer. Other volumes may be required if the interaction is difficult to break.
- 8 The purified fractions can be buffer exchanged using HiTrap Desalting, HiPrep 26/10 Desalting or PD-10 Desalting columns if necessary (Table 5).

Note: *The reuse of HiTrap Protein A HP depends on the nature of the sample and should only be performed with identical antibodies to prevent cross-contamination.*

Note: *If a P-1 pump is used a max flow rate of 1 to 3 ml/min can be run on a HiTrap 1 ml column packed with Sepharose HP media.*

Table 5. Prepacked columns for desalting and buffer exchange

Column	Loading volume	Elution volume
HiPrep 26/10 Desalting ¹	2.5 to 15 mL	7.5 to 20 mL
HiTrap Desalting ²	0.25 to 1.5 mL	1.0 to 2.0 mL
PD-10 Desalting ³	1.0 to 2.5 mL ⁴ 1.75 to 2.5 mL ⁵	3.5 mL Up to 2.5 mL
PD MiniTrap™ G-25	0.1 to 2.5 mL ⁴ 0.2 to 0.5 mL ⁵	1.0 mL Up to 0.5 mL
PD MidiTrap™ G-25	0.5 to 1 mL ⁴ 0.75 to 1 mL ⁵	1.5 mL Up to 1 mL

¹ Prepacked with Sephadex™ G-25 Fine and requires a pump or a chromatography system to run.

² Prepacked with Sephadex G-25 Superfine and requires a syringe or pump to run.

³ Prepacked with Sephadex G-25 and can be run by the gravity flow or centrifugation.

⁴ Volumes with gravity elution.

4 Scaling up

For quick scale-up of purification, two or three HiTrap columns can be connected in series (back pressure will increase).

If further scale-up is necessary bulk media packages of nProtein A Sepharose 4 Fast Flow are available.

5 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Fig 3. Increased pressure is generated when running/using one or a combination of the following conditions:

- High flow rates
- Buffers or sample with high viscosity
- Low temperature
- A flow restrictor

Note: *Exceeding the flow limit (see Table 2) may damage the column.*

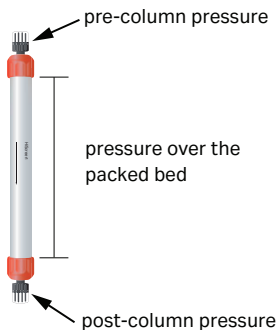


Fig 3. Pre-column and post-column measurements.

ÄKTA avant

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, Δp). The pre-column pressure limit is the column hardware pressure limit (see Table 1).

The maximum pressure the packed bed can withstand depends on media characteristics and sample/liquid viscosity. The measured value also depends on the tubing used to connect the column to the instrument.

ÄKTAexplorer, ÄKTApurifier, ÄKTAFLC and other systems with pressure sensor in the pump

To obtain optimal functionality, the pressure limit in the software may be adjusted according to the following procedure:

- 1 Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note the pressure as *total system pressure*, P1.
- 2 Disconnect the tubing and run the pump at the same flow rate used in step 1. Note that there will be a drip from the column valve. Note this pressure as P2.
- 3 Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see Table 1). Replace the pressure limit in the software with the calculated value.

The actual pressure over the packed bed (Δp) will during run be equal to actual measured pressure - *total system pressure* (P1).

Repeat the procedure each time the parameters are changed.

6 Storage

Before storage, we recommend to wash the column with 5 column volumes of 20% ethanol to prevent microbial growth. Store the column in 20% ethanol at 2°C to 8°C.

7 Ordering information

Product	Pack size	Code No.
HiTrap Protein A HP	1 × 1 ml	29-0485-76
	2 × 1 ml	17-0402-03
	5 × 1 ml	17-0402-01
	1 × 5 ml	17-0403-01
	5 × 5 ml	17-0403-03
Related products	Pack size	Code No.
HiTrap MabSelect™ SuRe™	1 × 1 ml	29-0491-04
	5 × 1 ml	11-0034-93
	1 × 5 ml	11-0034-94
	5 × 5 ml	11-0034-95
HiTrap MabSelect	5 × 1 ml	28-4082-53
	1 × 5 ml	28-4082-55
	5 × 5 ml	28-4082-56
HiTrap MabSelect Xtra™	5 × 1 ml	28-4082-58
	1 × 5 ml	28-4082-60
	5 × 5 ml	28-4082-61
HiTrap rProtein A FF	2 × 1 ml	17-5079-02
	5 × 1 ml	17-5079-01
	1 × 5 ml	17-5080-01
	5 × 5 ml	17-5080-02
HiTrap Protein G HP	1 × 1 ml	29-0485-81
	2 × 1 ml	17-0404-03
	5 × 1 ml	17-0404-01
	1 × 5 ml	17-0405-01
	5 × 5 ml	17-0405-03
MAbTrap™ Kit	1 kit	17-1128-01
HiTrap Desalting	1 × 5 ml	29-0486-84
	5 × 5 ml	17-1408-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01

Related products	Pack size	Code No.
	4 × 53 ml	17-5087-02
PD-10 Desalting Column	30	17-0851-01

Accessories	Quantity	Code No.
1/16" male/luer female <i>(For connection of syringe to top of HiTrap column)</i>	2	18-1112-51
Tubing connector flangeless/M6 female <i>(For connection of tubing to bottom of HiTrap column)</i>	2	18-1003-68
Tubing connector flangeless/M6 male <i>(For connection of tubing to top of HiTrap column)</i>	2	18-1017-98
Union 1/16" female/M6 male <i>(For connection to original FPLC System through bottom of HiTrap column)</i>	6	18-1112-57
Union M6 female /1/16" male <i>(For connection to original FPLC System through top of HiTrap column)</i>	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTA design	8	28-4010-81
Stop plug female, 1/16" <i>(For sealing bottom of HiTrap column)</i>	5	11-0004-64
Fingertight stop plug, 1/16"	5	11-0003-55

Literature	Code No.
Antibody Purification Handbook	18-1037-46
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography, Column and Media Selection Guide	18-1121-86

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