cytiva

HiTrap Protein G HP, 1 ml and 5 ml

Instructions for Use

HiTrap™ Protein G HP is a prepacked ready to use, column for 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 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.*

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 G HP 1 ml and 5 ml columns are packed with 1 ml and 5 ml of Protein G Sepharose™ High Performance, respectively. Protein G Sepharose High Performance is designed for purification and isolation of monoclonal and polyclonal IgG from ascites, serum and cell culture supernatants.

Protein G, a cell surface protein of Group G streptococci, is a Type III Fc receptor that binds to the Fc region of IgG by a non-immune mechanism similar to that of protein A of Staphylococcus aureus. Protein G and protein A, however, have different IgG binding specificities, dependent on the origin of the IgG. Compared with 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 (Table 3).

Our recombinant protein G, M_r 17 000, is produced in *E. coli* and contains two IgG binding regions. The albumin binding region of native protein G has been genetically deleted, thereby avoiding undesirable cross-reactions with albumin.

Synthesis of this medium involves coupling protein G 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 the matrix-bound protein G for IgG depends on the source species of the immunoglobulin. The total capacity depends also upon several other factors such as the flow rate during sample application, and the sample concentration. As a reference, its binding capacity for human IgG is approximately 25 mg IgG/ml medium.

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

Table 2. HiTrap Protein G HP characteristics

Ligand	Recombinant protein G lacking the albumin-binding region; M_r ~ 17 000, pI 4.1
Degree of substitution	~ 2 mg protein G/ml medium
Binding capacity	> 25 mg human IgG/ml medium
Mean particle size	34 µm
Bead structure	Highly cross-linked spherical agarose
Max. flow rates[1]	4 ml/min 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

2 Operation

Protein G binds IgG over a wide pH range and thus permits the selection of buffer pH relevant for any particular application. There is a strong affinity between protein G and IgG at pH 7.0. To elute the IgG it is necessary to lower the pH to about 2.5 to 3.0 depending on the sample.

As a safety measure to preserve the activity of acid labile IgGs, we recommend the addition of 60 to 200 µl of 1 M Tris-HCl, pH 9.0, to those tubes destined to collect fractions containing IgG.

In this way 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 buffer: 20 mM sodium phosphate, pH 7.0

Elution buffer: 0.1 M glycine-HCl, pH 2.7

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 HiTrap Desalting, HiPrep™ 26/10 Desalting or PD-10 Desalting columns, see Table 4.

The sample should be fully solubilized. We recommend centifugation or filtration immediately before loading on the column to remove particulate material (0.45 µm filter).

Never apply turbid solution to the column.

3 Purification

1. Prepare collection tubes by adding 60 to 200 µl 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 4).

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

Note: *If a P1-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 High Performance.*

4 Scaling up

For quick scale up of purifications, two or three HiTrap Protein G HP 1 ml or 5 ml column can be connected in series (backpressure will increase). Further scale-up can be achieved using bulk media packages of Protein G Sepharose 4 Fast Flow.

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.*

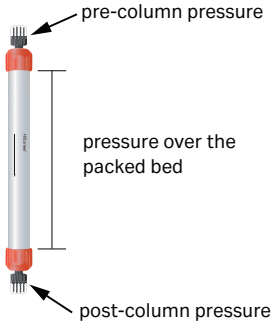

Fig 3. Pre-column and post-column measurements.

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, ÄKTAFPLC 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).

Note: *Repeat the procedure each time the parameters are changed.*

Table 4. 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.

[5] Volumes with centrifugation.

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	No. Supplied	Code No.
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

Related products	No. Supplied	Code No.
MAbTrap™ Kit	1 kit	17-1128-01
HiTrap MabSelect SuRe™	5 × 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 A HP	2 × 1 ml	17-0402-03
	5 × 1 ml	17-0402-01
	1 × 5 ml	17-0403-01
	5 × 5 ml	17-0403-03
HiTrap Desalting	1 × 5 ml	29-0486-84
	5 × 5 ml	17-1408-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02

Related products	No. Supplied	Code No.
PD-10 Desalting Column	30	17-0851-01

Accessories	Quantity	Code No.
1/16" male/luer female *(For connection of syringe to top of HiTrap column*)	2	18-1112-51
Tubing connector flangeless/M6 female (*For connection of tubing to bottom of HiTrap column)*	2	18-1003-68
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18-1017-98
Union 1/16" female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18-1112-57
Union M6 female /1/16" male *(For connection to original FPLC System through top of HiTrap column*)	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" *(For sealing bottom of HiTrap column)*	5	11-0004-64
Fingertight stop plug, 1/16"	5	11-0003-55

Related literature	Code No.
Antibody Purification Handbook	18-1037-46
Solutions for antibody purification, Selection Guide	28-9351-97
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography Columns and Media, Selection Guide	18-1121-86

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