GE Healthcare

Instructions 71-7002-00 AP **HiTrap affinity columns**

HiTrap Protein A HP, 1 ml and 5 ml

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™ design.

HiTrap

Code No.	Product	No. supplied
17-0402-01	HiTrap Protein A HP	5 × 1 ml
17-0402-03	HiTrap Protein A HP	2 × 1 ml
17-0403-01	HiTrap Protein A HP	1 × 5 ml
17-0403-03	HiTrap Protein A HP	5 × 5 ml

Connector kit

Connectors supplied	Usage	No. supplied
1/16" male/luer female	Connection of syringe to top of HiTrap column	1
Tubing connector flangeless/ M6 female	Connection of tubing (e.g. Peristaltic Pump P1) to bottom of HiTrap column[1]	1
Tubing connector flangeless/M6 male	Connection of tubing (e.g. Peristaltic Pump P1) to top of HiTrap column[2]	1
Union 1/16" female/M6 male	Connection to original FPLC™ System through bottom of HiTrap column	1
Union M6 female/ 1/16" male	Connection to original FPLC System through top of HiTrap column	1
Stop plug female, 1/16"	Sealing bottom of HiTrap column	2, 5 or 7

[1] Union 1/16" female/M6 male is also needed.
[2] Union M6 female/1/16" male is also needed.

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1 Description

Column

HiTrap Protein A HP 1 ml and 5 ml columns are made of polypropylene, which is biocompatible and non-interactive with biomolecules. The top and bottom frits are manufactured from porous polyethylene. It is delivered with a stopper on the inlet and a snap-off end on the outlet.

The separation can be easily achieved using a syringe together with the supplied luer adaptor, a peristaltic pump, or in a chromatography system such as ÄKTA design.

Note: *To prevent leakage it is essential to ensure that the connector is tight*.

The column cannot be opened or refilled.

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 2).

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 Tables 2 and 3. 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 1.

Table 1. HiTrap Protein A HP characteristics

Column volumes	1 ml and 5 ml
Column dimensions	0.7 × 2.5 cm (1 ml) and 1.6 × 2.5 cm (5 ml)
Ligand	Protein A, M_r ~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	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[1]	
long term	3 to 9
short term	2 to 9
Storage	4°C to 8°C in 20% ethanol

[1] 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 2. 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 3. 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 60–200 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,
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 4. 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 60–200 µl 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 5–10 column volumes of binding buffer or until no material appears in the effluent.
7. Elute with 2–5 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 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 1–3 ml/min can be run on a HiTrap 1 ml column packed with Sepharose HP media.*

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 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 4°C to 8°C.

6 Intended use

The HiTrap Protein A HP is intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Table 4. Prepacked columns for desalting and buffer exchange

Column	Code No.	Loading volume	Elution volume	Comments	Application
HiPrep 26/10 Desalting	17-5087-01	2.5–15 ml	7.5–20 ml	Prepacked with Sephadex™ G-25 Fine. Requires a laboratory pump or a chromatography system to run.	For desalting and buffer exchange of protein extracts (M_r > 5000).
HiTrap Desalting	17-1408-01	0.25–1.5 ml	1.0–2.0 ml	Prepacked with Sephadex G-25 Superfine. Requires a syringe or pump to run.	
PD-10 Desalting	17-0851-01	1.0–2.5 ml[1] 1.75–2.5 ml[2]	3.5 ml[1] Up to 2.5 ml[2]	Prepacked with Sephadex G-25 Medium. Runs by gravity flow or centrifugation	For desalting, buffer exchange, and cleanup of proteins and other large biomolecules (M_r > 5000).
PD MiniTrap™ G-25	28-9180-07	0.1– 0.5 ml[1] 0.2–0.5 ml[2]	1.0 ml[1] Up to 0.5 ml[2]		
PD MidiTrap™ G-25	28-9180-08	0.5–1.0 ml[1] 0.75–1.0 ml[2]	1.5 ml[1] Up to 1.0 ml[2]		

[1] Volumes with gravity elution
[2] Volumes with centrifugation

7 Ordering information

Product	Pack size	Code No.
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

Related products	Pack size	Code No.
HiTrap MabSelect SuRe™	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	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	5 × 5 ml	17-1408-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02
PD-10 Desalting Column	30	17-0851-01

Accessories	No. Supplied	Code No.
1/16" male/luer female[1]	2	18-1112-51
Tubing connector flangeless/ M6 female[1]	2	18-1003-68
Tubing connector flangeless/M6 male[1]	2	18-1017-98
Union 1/16" female/M6 male[1]	6	18-1112-57
Union M6 female /1/16" male[1]	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"[2]	5	11-0004-64
Fingertight stop plug, 1/16"[3]	5	11-0003-55

[1] One connector included in each HiTrap package.
[2] Two, five, or seven stop plugs female included in HiTrap packages depending on the product.
[3] One fingertight stop plug is connected to the top of each HiTrap column at delivery.

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
HiTrap Column Guide	18-1129-81

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71-7002-00 AP 02/2009