Instructions 71-5002-73 AK

HiTrap™ rProtein A FF, 1 ml and 5 ml

HiTrap rProtein A FF is a prepacked ready to use, column for easy purification of antibodies.

The special design of the column, together with the matrix, provide fast, simple and easy separations in a convenient format.

HiTrap rProtein A FF offers a way to purify antibodies like monoclonals from ascites and cell culture supernatants.

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

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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.

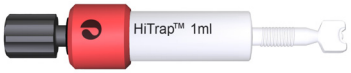

Fig 1. HiTrap, 1 ml column.

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

rProtein A Sepharose™ Fast Flow is designed for purification and isolation of monoclonal antibodies from ascites and cell culture supernatants. The ligand has been specially engineered to give very high binding capacities.

The specificity of protein A is primarily for the Fc region of IgG. However, it can also bind the Fab region through secondary sites. There are differences between the binding affinities for Fc and Fab, usually Fc binding is stronger, which can provide a means of fractionating Fab or $F(ab)_2$ from Fc.

The characteristics of the products are summarized in Table 2.

The degree to which protein A binds to IgG varies with respect to both origin and antibody subclass and may even vary substantially within a single subclass, see Tables 3 and 4. The binding capacity of protein A for IgG depends on the source species of the particular immunoglobulin. The total capacity depends also upon several other factors such as the flow rate during sample application, and the sample concentration. This gel has a binding capacity for human IgG of approximately 50 mg IgG/ml medium.

The ligand rProtein A is coupled to highly cross-linked 4% agarose beads by a technique which generates a stable thioether linkage between rProtein A and the base matrix. The coupling technique is optimized to give a high binding capacity for IgG.

Table 2. HiTrap rProtein A FF characteristics

Ligand	recombinant protein A, (*E. coli*)
Degree of substitution	~ 6 mg rProtein A/ml medium
Total binding capacity	~ 50 mg human IgG/ml medium
Dynamic binding capacity[1]	23 mg mouse monoclonal IgG_{2a}/ml medium 12 mg mouse monoclonal IgG_1/ml medium 11 mg monoclonal humanized IgG_4/ml medium
Average particle size	90 µm
Bead structure	Highly cross-linked 4% agarose
Maximum flow rate[2]	4 ml/min (624 cm/h) and 20 ml/min (600 cm/h) for 1 and 5 ml columns respectively
Recommended working flow rate	1 ml/min (156 cm/h) and 5 ml/min (150 cm/h) for 1 and 5 ml columns respectively
Chemical stability	All commonly used buffers
pH stability[3]	
Short term	2 to 11
Long term	3 to 10
Storage	2°C to 8°C in 20% ethanol

[1] Capacity of HiTrap rProtein A for some monoclonal antibodies. Running conditions: Binding buffer: 20 mM sodium phosphate (+3 M NaCl for IgG_1), pH 7.0, Elution buffer: 0.1 M sodium citrate, pH 3.0. Column: HiTrap rProtein A FF 1 ml. Flow rate: 1 ml/min (156 cm/h). Sample: cell culture supernatants.

[2] Room temperature, aqueous buffers.

[3] pH below 3 is sometimes required to elute strongly bound Ig's. However, protein ligands may hydrolyze at very low pH.
pH stability, Short term, refers to the pH interval for regeneration and cleaning.
pH stability, Long term, refers to the pH interval where the medium is stable over a long period without adverse effects on its subsequent chromatographic performance.

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.

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

Table 4. 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

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 sodium citrate, pH 3–6

With some antibodies, e.g., mouse IgG_1, it might be necessary to add sodium chloride up to 4 M in the binding buffer, to achieve efficient binding.

High salt binding buffer: 1.5 M glycine, 3 M NaCl, pH 8.9 or 20 mM sodium phosphate, 3 M NaCl, pH 7.0

Elution buffer: 0.1 M sodium citrate, pH 3–6

As a safety measure to preserve the activity of acid labile IgG when using very acidic elution conditions, we recommend adding 60 to 200 µl of 1 M Tris-HCl, pH 9.0 per ml of eluted fraction to be collected, so that the final pH of the sample will be approximately neutral.

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 5. The sample should be filtered through a 0.45 µm filter or centrifuged immediately before it is applied to the column. Never apply a turbid solution to the column. (This is especially important to prevent clogging of column when loading large volumes of serum or plasma).

3 Purification

We recommend to use a flow rate of 1 ml/min for HiTrap rProtein A FF 1 ml column and 5 ml/min for HiTrap rProtein A FF 5 ml column.

1 Prepare collection tubes by adding 60 to 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 out the ethanol preservative with at least 5 column volumes of distilled water or binding buffer.

5 Regenerate the column with 5 column volumes of elution buffer.

6 Equilibrate the column with 5 to 10 column volumes of binding buffer.

7 Apply the sample, using a syringe fitted to the luer connector or by pumping it onto the column.

8 Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent. Excessive washing should be avoided if the interaction between the protein of interest and the ligand is weak, since this may decrease the yield.

9 Elute with elution buffer. 2 to 5 column volumes is usually sufficient, but other volumes (or different elution buffer) will be required if the interaction is difficult to break.

10 The purified IgG fractions can be buffer exchanged using

11 HiTrap Desalting, HiPrep 26/10 Desalting or PD-10 Desalting columns if necessary (Table 5).

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

Table 5. 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

4 Scaling up

HiTrap rProtein A FF columns can be connected in series if even higher capacities are required (back pressure will increase). Further scale up can be done using bulk media packs.

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.*

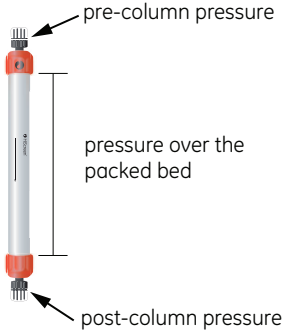

Fig 3. Pre-column and post-column measurements.

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.*

6 Storage

Before storage, we recommend to wash the column with 5 column volumes of 20% ethanol to prevent microbial growth. Seal the column with the supplied stoppers. Store the HiTrap rProtein A FF column at 2°C to 8°C.

7 Ordering information

Product	Pack size	Code No.
HiTrap rProtein A FF	2 × 1 ml	17-5079-02
	5 × 1 ml	17-5079-01
	100 × 1 ml*	28-9464-89
	1 × 5 ml	17-5080-01
	5 × 5 ml	17-5080-02

* Special pack size delivered on specific customer request.

Related products	Pack size	Code No.
rProtein A Sepharose Fast Flow	5 ml	17-1279-01
	25 ml	17-1279-02
	200 ml	17-1279-03
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 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
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
HiTrap MAb 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
	4 × 53 ml	17-5087-02
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 & Methods	18-1022-29
Affinity Chromatography Columns and Media, Selection Guide	18-1121-86

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