Instructions 71500273 AL

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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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, 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 resin, 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

Resin 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 resin has a binding capacity for human IgG of approximately 35 mg IgG/mL resin.

The ligand rProtein A is coupled to 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

Matrix	cross-linked agarose, 4%, spherical
Particle size, d_{50v}[1]	~ 90 µm
Ligand	Recombinant protein A, (*E. coli*)
Dynamic binding capacity[2]	~ 35 mg human IgG/mL resin
Dynamic binding capacity[3]	~ 23 mg mouse monoclonal IgG_{2a}/mL resin ~ 12 mg mouse monoclonal IgG_1/mL resin ~ 11 mg monoclonal humanized IgG_4/mL resin
Maximum operating flow rate[4]	1 mL column: 4 mL/min 5 mL column: 20 mL/min
Recommended operating flow rate[4]	1 mL column: 0.5 mL/min 5 mL column: 2.5 mL/min
Chemical stability	Stable to commonly used aqueous buffers, 6 M guanidine hydrochloride, 2% benzyl alcohol, 1 mM NaOH (pH 11), 0.1 M sodium citrate/HCL (pH3), 20% ethanol
pH stability, operational[5] pH stability, CIP[7]	3 to 10[6] 3 to 12[6, 8]
Temperature stability	2°C to 40°C
Storage	20% ethanol, 2°C to 8°C

[1] Median particle size of the cumulative volume distribution.

[2] Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 100 cm/h in a 7.5/50 PEEK-column at 5 cm bed height (3 min residence time) for human IgG in 0.020 M NaH_2PO_4, pH 7.0

[3] 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 supernatnants.

[4] At room temperature using buffers with the same viscosity as water.

[5] pH range where resin can be operated without significant change in function.

[6] pH below 3 is sometime required to elute strongly bound IgG species. However, protein ligands may hydrolyze at pH below 2.

[7] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

[8] Reducing agent e.g., 100 mM 1-thioglycerol followed by 15 mM NaOH is among the most efficient CIP for rProtein Sepharose Fast Flow.

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 must 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 to 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 to 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 0.5 mL/min for HiTrap rProtein A FF 1 mL column and 2.5 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 Remove the stopper from the inlet and the snap-off end at the column outlet.

3 Connect the column to the system with 1/16" male connectors (28401081).

Note: *Make a drop-to-drop connection to prevent air from entering the column. Make sure that the connections are tight to prevent leakage.*

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 might 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 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.

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.

4 Scaling up

HiTrap rProtein A FF columns can be connected in series if even higher capacities are required (backpressure will increase). Further scale-up can be done using bulk BioProcess™ resin packs.

BioProcess chromatography resins are developed and supported for production scale chromatography. BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

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 Figure 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) might damage the column.*

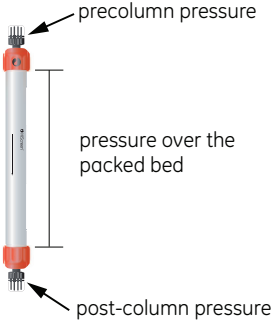

Fig 3. Precolumn and post-column measurements.

ÄKTA avant and ÄKTA pure

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

The maximum pressure the packed bed can withstand depends on resin 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 can 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	Product code
HiTrap rProtein A FF	2 × 1 mL	17507902
	5 × 1 mL	17507901
	1 × 5 mL	17508001
	5 × 5 mL	17508002

Related products	Pack size	Product code
rProtein A Sepharose Fast Flow	5 mL	17127901
	25 mL	17127902
	200 mL	17127903
HiTrap MabSelect™ PrismA	1 × 1 mL	17549851
	5 × 1 mL	17549852
	1 × 5 mL	17549853
	5 × 5 mL	17549854
HiTrap MabSelect SuRe™	1 × 1 mL	29049104
	5 × 1 mL	11003493
	1 × 5 mL	11003494
	5 × 5 mL	11003495
HiTrap Protein A HP	1 × 1 mL	29048576
	2 × 1 mL	17040203
	5 × 1 mL	17040201
	1 × 5 mL	17040301
	5 × 5 mL	17040303
HiTrap Protein G HP	1 × 1 mL	29048581
	2 × 1 mL	17040403
	5 × 1 mL	17040401
	1 × 5 mL	17040501
	5 × 5 mL	17040503
HiTrap MAb kit	1 kit	17112801
HiTrap Desalting	1 × 5 mL	29048684
	5 × 5 mL	17140801
HiPrep 26/10 Desalting	1 × 53 mL	17508701
	4 × 53 mL	17508702
PD-10 Desalting Column	30	17085101

Accessories	Quantity	Product code
1/16" male/luer female *(For connection of syringe to top of HiTrap column)*	2	18111251
Tubing connector flangeless/M6 female (*For connection of tubing to bottom of HiTrap column)*	2	18100368
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18101798
Union 1/16" female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18111257
Union M6 female /1/16" male *(For connection to original FPLC System through top of HiTrap column*)	5	18385801
Union luerlock female/M6 female	2	18102712
HiTrap/HiPrep, 1/16" male connector for ÄKTA design	8	28401081
Stop plug female, 1/16" *(For sealing bottom of HiTrap column)*	5	11000464
Fingertight stop plug, 1/16"	5	11000355

Related literature	Product
Antibody Purification Handbook	18103746
Solutions for antibody purification, Selection Guide	28935197
Affinity Chromatography Handbook, Principles & Methods	18102229
Affinity Chromatography Columns and Media, Selection Guide	18112186

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71500273 AL 09/2018