HiTrap rProtein A FF

HiTrap™ rProtein A FF are 1 ml and 5 ml prepacked columns that allow quick, easy, and convenient purification of polyclonal antibodies and monoclonal antibodies from cell culture supernatants, serum, and ascites.

Features

- Rapid and convenient preparative purification of polyclonal and monoclonal antibodies
- Specially engineered ligand to give enhanced binding capacities
- Simple and proven method giving reproducible results
- Easy to use with syringe, pump or chromatographic system such as ÄKTA™design or FPLC™ System

Introduction

The specificity of the recombinant protein A for the Fc region of IgG is similar to native protein A and provides excellent purification in one step.
The degree to which protein A binds to IgG varies with respect to both origin and antibody subclass and may even vary within a single subclass. The binding capacity of protein A for IgG depends on the source species of the particular immunoglobulin. The capacity depends also upon several other factors such as flow rate during sample application, and sample concentration.

Medium characteristics

Recombinant *(E. coli)* protein A ligand is coupled to Sepharose™ Fast Flow by a technique which generates a stable thioether linkage between rProtein A and the base matrix. The coupling technique is optimized to give high binding capacity for IgG, see Figure 2.

Column characteristics

HiTrap rProtein A FF 1 ml and 5 ml columns are packed with 1 ml and 5 ml of rProtein A Sepharose Fast Flow, respectively. The columns are made of medical grade polypropylene, are biocompatible and do not interact with biomolecules. Columns have porous top and bottom frits which allow high flow rates. Both ends have M6 connections (6 mm metric threads). Connectors are included for connection to ÄKTAdesign systems.

Fig 1. HiTrap rProtein A FF for convenient purification of polyclonal and monoclonal antibodies.

Sample:	30 ml myeloma cell culture containing humanised IgG_4, filtered through a 0.45 µm filter. The sample was a kind gift from Dr. J. Bonnerjea, LONZA Biologics plc, UK
Columns:	HiTrap rProtein A FF 1 ml Supplier B 1 ml
Binding buffer:	0.02 M sodium phosphate, pH 7.0
Elution buffer:	0.1 M sodium citrate, pH 3.0
Flow rates:	1 ml/min (156 cm/h) on HiTrap rProtein A FF 1 ml 0.7 ml/min (140 cm/h) on Supplier B 1 ml (recommended by manufacturer)
Instrumentation:	FPLC™ System

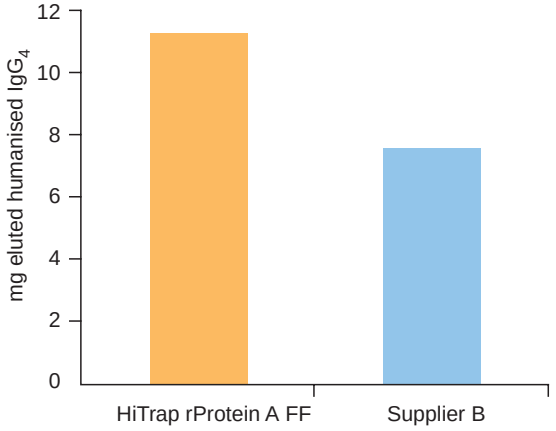


Fig 2. Comparison of binding capacity of humanized IgG_4 on HiTrap rProtein A FF 1 ml column and Supplier B 1 ml column.

Amersham Biosciences

Column volumes	1 ml and 5 ml
Column dimensions, i.d. × h	0.7 × 2.5 cm (1 ml) and 1.6 × 2.5 cm (5 ml)
Ligand	recombinant protein A, *(E. coli)*
Degree of substitution	≈ 6 mg rProtein A/ml gel
Total binding capacity	≈ 50 mg human IgG/ml gel
Dynamic binding capacities*	23 mg mouse monoclonal IgG_{2a}/ml gel 12 mg mouse monoclonal IgG_1/ml gel 11 mg monoclonal humanized IgG_4/ml gel
Mean particle size	90 µm
Bead structure	highly cross-linked 4% agarose
Recommended flow rate	1 ml/min (156 cm/h) and 5 ml/min (150 cm/h) for 1 and 5 ml columns, respectively
Maximum flow rate**	4 ml/min (624 cm/h) and 20 ml/min (600 cm/h) for 1 and 5 ml columns, respectively
Maximum back pressure	3 bar, 0.3 MPa
Chemical stability	All commonly used buffers
pH stability***	
Long term	3–10
Short term	2–11
Temperature stability	
Regular use	+4 ° to +40 °C
Storage	+4 ° to +8 °C
Storage buffer	20% ethanol

* Capacity of HiTrap rProtein A FF 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.
Sample: Monoclonal cell culture supernatants

** Room temperature, aqueous buffers

*** 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 gel 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 cleaning.
Protein A may hydrolyze at low pH. Complete data on the stability of rProtein A as a function of pH are not available.

Table 1. HiTrap rProtein A FF characteristics.

Operation

As for all HiTrap columns, HiTrap rProtein A FF is quick and convenient to use. Instructions and connectors are included with each pack of columns. In general, separation can easily be achieved with a syringe (using the luer adaptor provided). Figure 3 a–c illustrates this technique. Alternatively, the column can be operated using a laboratory pump or a chromatography system when linear gradients are required or when large sample volumes are loaded.

HiTrap rProtein A FF columns can easily be connected in series if even higher capacities are required.

The columns cannot be opened or repacked.

The characteristics of HiTrap rProtein A FF are summarized in Table 1.

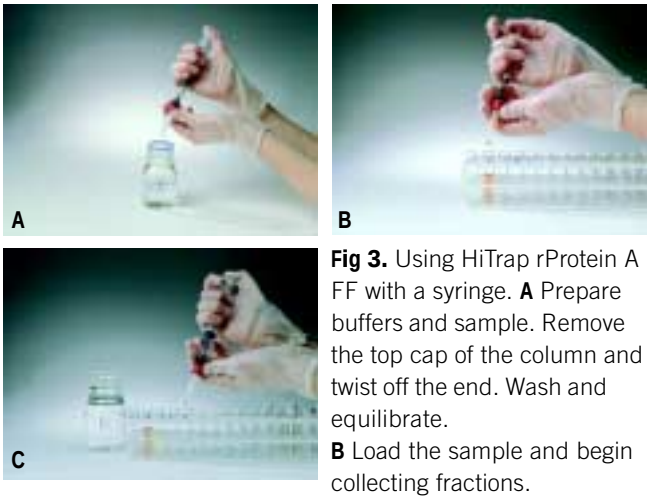


Fig 3. Using HiTrap rProtein A FF with a syringe. **A** Prepare buffers and sample. Remove the top cap of the column and twist off the end. Wash and equilibrate.
B Load the sample and begin collecting fractions.
C Wash, elute and continue collecting fractions.

Applications

Purification of monoclonal mouse IgG_{2b} from ascites

Mouse IgG_{2b} was purified on HiTrap rProtein A FF 1 ml column operated with a syringe, (Fig 4). The eluted pool contained 1 mg IgG_{2b}.

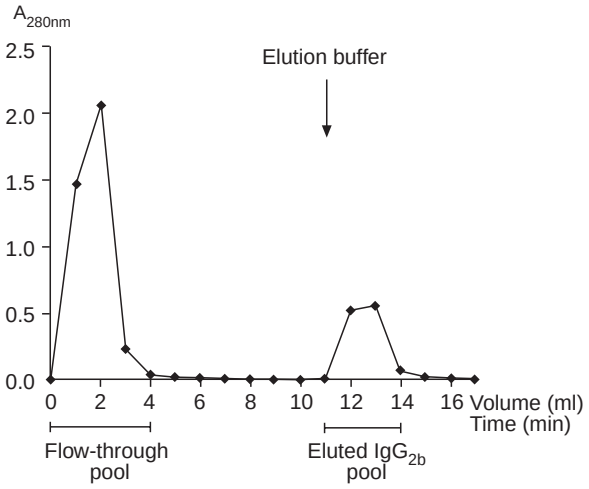
The silver-stained SDS-PAGE gel confirmed that the eluted antibody was over 95% pure, see Figure 4.

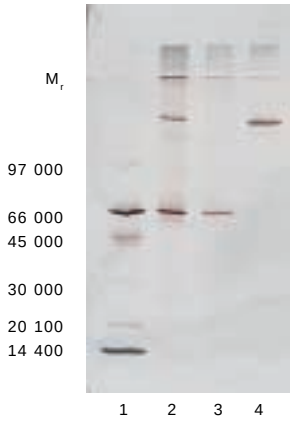
Purification of monoclonal mouse IgG_1 from cell culture supernatant

Mouse IgG_1 was purified from 150 ml cell culture supernatant on HiTrap rProtein A FF 5 ml column. The purification was achieved using FPLC System (Fig 5). The eluted pool contained 28 mg IgG_1.

The eluted IgG_1 was over 95% pure according to SDS-PAGE with silver-staining, see Figure 5.

Sample: 1 ml mouse ascites containing IgG_{2b}, filtered through a 0.45 µm filter. The sample was a kind gift from Dr. N. Linde, EC Diagnostics, Sweden
Column: HiTrap rProtein A FF 1 ml
Binding buffer: 0.02 M sodium phosphate, pH 7.0
Elution buffer: 0.1 M sodium citrate, pH 3.0
Flow rate: ~ 1 ml/min
Instrumentation: Syringe

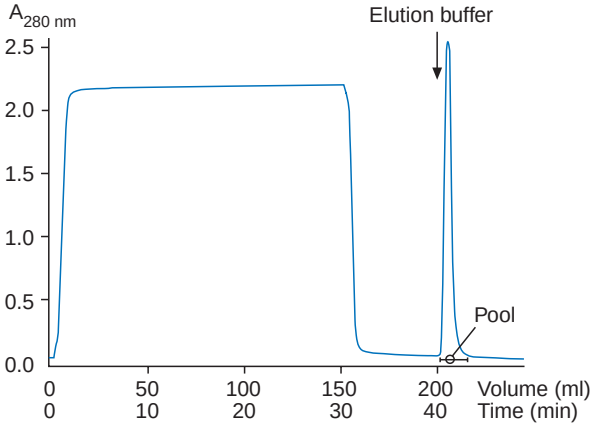


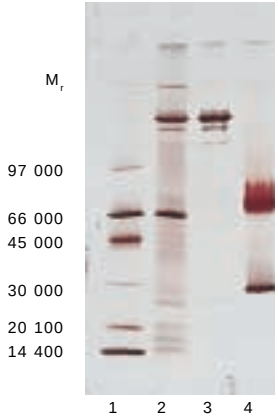


SDS-PAGE on PhastSystem™ using PhastGel™ Gradient 10–15, silver-staining
Lane 1: Low Molecular Weight Calibration Kit (LMW)
Lane 2: Starting material, mouse ascites, dil. 10×
Lane 3: Flow-through pool
Lane 4: Eluted IgG_{2b} pool

Fig 4. Purification of mouse IgG_{2b} from ascites on HiTrap rProtein A FF 1 ml column using a syringe.

Sample: 150 ml of cell culture supernatant containing IgG_1, filtered through a 0.45 µm filter.
Column: HiTrap rProtein A FF 5 ml
Binding buffer: 0.02 M sodium phosphate, 3 M NaCl, pH 7.0
Elution buffer: 0.1 M sodium citrate, pH 3.0
Flow rate: 5 ml/min (150 cm/h)
Instrumentation: FPLC System





SDS-PAGE on PhastSystem using PhastGel Gradient 10–15, silver-staining
Lane 1: LMW
Lane 2: Starting material, cell culture supernatant
Lane 3: Eluted IgG_1 pool, dil. 5×
Lane 4: Eluted IgG_1 pool, dil. 5×, reduced

Fig. 5. Purification of mouse IgG_1 from cell culture supernatant on HiTrap rProtein A FF 5 ml column.

Immunoglobulin	Heavy chain	Light chain	Sedimentation coefficient	Mol. Wt (M_r)	M_r heavy chain	Carbohydrate content (%)	A_{280nm}	pI
IgG_1	λ_1	κ,λ	7S	146 000	50 000	2–3	13.8	5.0–9.5
IgG_2	λ_1	κ,λ	7S	146 000	50 000	2–3		5.0–8.5
IgG_3	λ_1	κ,λ	7S	170 000	60 000	2–3		8.2–9.0
IgG_4	λ_1	κ,λ	7S	146 000	50 000	2–3		5.0–6.0
IgM	μ	κ,λ	19S	900 000	68 000	12	12.5	5.1–7.8
IgA_1	α_1	κ,λ	7S	160 000	56 000	7–11	13.4	5.2–6.6
IgA_2	α_2	κ,λ	7S	160 000	52 000	7–11		5.2–6.6
IgA_s	α_1, α_2	κ,λ	11S	370 000	52–56 000	11		4.7–6.2
IgD	δ	κ,λ	7S	184 000	68 000	12	17.0	–
IgE	ε	κ,λ	8S	190 000	72 000	12	15.3	–

Table 2. Physio-chemical properties of human immunoglobulins.

Immunoglobulin	Heavy chain	Light chain	Sedimentation coefficient	Mol. Wt (M_r)	M_r heavy chain	Carbohydrate content (%)	pI
IgG_1	λ_1	κ,λ	7S	150 000	50 000	2–3	7.0–8.5
IgG_{2a}	λ_{2a}	κ,λ	7S	150 000	50 000	2–3	6.5–7.5
IgG_{2b}	λ_{2b}	κ,λ	7S	150 000	50 000	2–3	5.5–7.0
IgG_3	λ_3	κ,λ	7S	150 000	50 000	2–3	–
IgM	μ	κ,λ	19S	900 000	80 000	12	4.5–7.0
IgA	α	κ,λ	7S	170 000	70 000	7–11	4.0–7.0
IgD	δ	κ,λ	7S	180 000	68 000	12–14	–
IgE	ε	κ,λ	8S	190 000	80 000	12	–

Table 3. Physio-chemical properties of mouse immunoglobulins.

Ordering information

Product	Quantity	Code No.
HiTrap rProtein A FF, 1 ml	5 × 1 ml	17-5079-01
HiTrap rProtein A FF, 1 ml	2 × 1 ml	17-5079-02
HiTrap rProtein A FF, 5 ml	1 × 5 ml	17-5080-01
rProtein A Sepharose Fast Flow	5 ml	17-1279-01
rProtein A Sepharose Fast Flow	25 ml	17-1279-02
rProtein A Sepharose Fast Flow	200 ml	17-1279-03
HiTrap Desalting, 5 ml	5 × 5 ml	17-1408-01
Accessories		
Domed nut *	4	18-2450-01
Union Luerlock adaptors		
female/M6 male *	2	18-1027-62
female/M6 female *	2	18-1027-12
Tubing connectors		
flangeless/M6 male *	2	18-1017-98
flangeless/M6 female *	2	18-1003-68
Union 1/16″ female/M6 male *	6	18-1112-57
Union M6 female/1/16″ male *	8	18-1112-58
Technical information		
Antibody Purification Handbook	1	18-1037-46
Affinity Chromatography Handbook	1	18-1022-29
Principles and Methods		
Convenient Protein Purification		
HiTrap Column Guide	1	18-1129-81

* Included in HiTrap package

to order:

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