HiTrap rProtein A FF
HiTrap Protein A HP
HiTrap Protein G HP

AFFINITY CHROMATOGRAPHY

HiTrap™ rProtein A FF, HiTrap Protein A HP, and HiTrap Protein G HP are part of the range of prepacked, ready to use columns for preparative affinity chromatography. Fast, simple, and easy separations are provided by the combination of the easy-to-use HiTrap column and various affinity resins.

HiTrap rProtein A FF, HiTrap Protein A HP, and HiTrap Protein G HP 1 mL and 5 mL columns allow convenient purification of polyclonal and monoclonal antibodies from cell culture supernatants, serum, and ascites.

- Rapid and convenient preparative purification of polyclonal and monoclonal antibodies
- Very high purity in one step
- High binding capacities
- Simple and proven method giving reproducible results
- Simple operation with a syringe, a pump, an ÄKTA™ system, or other chromatography systems

The basis for antibody purification is the high affinity and specificity of protein A and protein G for the Fc-region of IgG from a variety of species. Protein A and protein G have been immobilized to several matrices resulting in excellent purification of IgG and IgG subclasses from ascites fluid, cell culture supernatants, and serum.

The degree to which protein A and protein G bind to IgG varies with respect to both origin and antibody subclass and might even vary within a single subclass. The binding capacity of protein A and protein G for IgG depends on the source species of the particular immunoglobulin. The capacity also depends upon several other factors such as flow rate during sample application and sample concentration.

Fig 1. nProtein A Sepharose 4 Fast Flow is available in a range of pack sizes.

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 (Table 2).

Column characteristics

HiTrap column is made of polypropylene, a material which is biocompatible and does not interact with biomolecules. The column is delivered with a stopper on the inlet and a snap-off end on the outlet. Both ends have 1/16" fittings for easy connection to ÄKTA systems.

Resin characteristics

rProtein A Sepharose Fast Flow

Recombinant protein A (rProtein A) and protein A share similar specificity for the Fc-region of IgG, but rProteinA offers several potential advantages. Since rProtein A has been engineered to include a C-terminal cysteine, controlled epoxy chemistry is used to favor single point oriented immobilization via thioether coupling and results in enhanced binding capacity for IgG. Furthermore, rProtein A is produced in *E. coli* and no human IgG affinity step is used during validated fermentation and purification processes, minimizing risk of human IgG contamination.

rProtein ligand is immobilized to Sepharose™ Fast Flow, a robust cross-linked agarose with spherical ~ 90 µm particles.

Protein A Sepharose High Performance and Protein G Sepharose High Performance

Sepharose High Performance is the base matrix for HiTrap Protein A HP and HiTrap Protein G HP. The carbohydrate in the agarose base provides a hydrophilic and chemically favorable environment for coupling, while the cross-linked structure of the ~ 34 µm spherical particles ensures excellent chromatographic properties. The protein A and protein G ligands are coupled to Sepharose High Performance by the N-hydroxysuccinimide activation method.

Protein A is a 42 000 molecular weight protein derived from a strain of *Staphylococcus aureus*. It consists of six regions, five of which bind IgG. As an affinity ligand, protein A is immobilized to the matrix so that these regions are free to bind. One molecule of immobilized protein A can bind at least two molecules of IgG.

Protein G, a cell surface protein from group G *Streptococci,* is a type III Fc receptor and binds IgG with a non-immune mechanism similar to that of protein A. Here a recombinant form of the protein produced in *E. coli,* from which the albumin-binding region of the native protein has been genetically deleted, is used. Recombinant protein G contains two Fc-binding regions.

Fast kinetics with high dynamic capacities are properties of all HiTrap affinity columns. The binding capacity of rProtein A, protein A and protein G for IgG depends on the source species of the particular immunoglobulin. The total capacity also depends upon several other factors, such as flow rate during sample application and sample concentration. As a reference, the total binding capacity for human IgG is approximately 20 mg IgG/mL resin for HiTrap Protein A HP and approximately 25 mg IgG/mL resin for HiTrap Protein G HP, and the dynamic binding capacity for HiTrap rProtein A FF is approximately 35 mg/mL resin.

Table 1 lists the main characteristics of HiTrap rProtein A FF, HiTrap Protein A HP and HiTrap Protein G HP.

Operation

HiTrap columns are quick and easy to use. Instructions and connectors are included with each pack of columns. In general, the separation can be easily achieved with a syringe, using the luer adapter provided. Figure 2 illustrates this technique. Alternatively, the column can be operated using a laboratory pump or a chromatography system when linear gradients are required or large sample volumes are loaded. Two or more columns can be connected in series by screwing the end of one into the top of the next (back pressure will increase). The columns cannot be opened or repacked.

Table 1. Main characteristics of HiTrap rProtein A FF, HiTrap Protein A HP and HiTrap Protein G HP

Column volumes	1 mL and 5 mL
Column dimensions	0.7 × 2.5 cm (1 mL) 1.6 × 2.5 cm (5 mL)
Ligand	Recombinant protein A (*E. coli*), protein A or protein G
Binding capacity (Approx.)	Dynamic binding capacity, Q_{B10}[1]: ~ 35 mg human IgG/mL resin (HiTrap rProtein A FF)
Total binding capacity HiTrap Protein A HP[2] HiTrap Protein G HP[3]	 ~ 20 mg human IgG/mL resin ~ 25 mg human IgG/mL resin
Dynamic binding capacities[4] (HiTrap rProtein A FF)	23 mg mouse monoclonal IgG_{2a}/mL resin 12 mg mouse monoclonal IgG_1/mL resin 11 mg monoclonal humanized IgG_4/mL resin
Particle size, d_{50V}[5]	~ 90 µm (HiTrap rProtein A FF) ~ 34 µm (HiTrap Protein A HP and HiTrap Protein G HP)
Matrix HiTrap rProtein A FF HiTrap Protein A HP and HiTrap Protein G HP	 Cross-linked agarose, 4%, spherical Cross-linked agarose, spherical
Recommended operating flow rate	1 and 5 mL/min for 1 and 5 mL columns, respectively
Maximum operating flow rate	4 and 20 mL/min for 1 and 5 mL columns, respectively
Column hardware pressure limit	0.5 MPa (5 bar, 70 psi)
pH stability, operational[6]	3 to 10[7] (HiTrap rProtein A FF) 3 to 9[7] (HiTrap Protein A HP and HiTrap Protein G HP)
pH stability, CIP[8]	3 to 12[9,7] (HiTrap rProtein A FF) 2 to 10[9,7] (HiTrap Protein A HP) 2 to 9[9,7] (HiTrap Protein G HP)
Storage	2°C to 8°C in 20% ethanol

[1] 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

[2] Protein in excess is loaded in 0.07 M NaCl with 0.27 M glycine at pH 7.0 on a Tricorn™ 5/50 GL column. The binding capacity is obtained by measuring the amounts of bound and eluted protein in 0.1 M glycine at pH 3.0.

[3] Protein in excess is loaded in 20 mM sodium phosphate at pH 7.0 on a Tricorn 5/50 GL column. The binding capacity is obtained by measuring the amounts of bound and eluted protein in 0.1 M glycine at pH 2.7.

[4] Running conditions for determining the dynamic binding capacity of HiTrap rProtein A FF:
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

[5] Median particle size of the cumulative volume distribution

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

[7] pH below 3 is sometimes required to elute strongly bound IgG species; however, protein ligands may hydrolyze at pH below 2.

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

[9] A reducing agent, such as 100 mM 1-thioglycerol, followed by 15 mM NaOH is among the most efficient CIP.

(A)

(B)

(C)

Fig 2. Using HiTrap rProtein A FF, HiTrap Protein A HP or HiTrap Protein G HP with a syringe. (A) Prepare buffers and sample. Remove the column's top cap and snap off the end. Wash and equilibrate; (B) Load the sample and begin collecting fractions; (C) Elute and continue collecting fractions.

Applications

Protein A and protein G have different IgG binding specificities, depending 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 rat IgG, human IgG_3, and mouse IgG_1. Table 2 lists the relative binding strengths of polyclonal IgG from various species to protein G and protein A. Binding was measured using a competitive ELISA test. The amount of IgG required to give a 50% inhibition of binding of rabbit IgG conjugated with alkaline phosphatase was determined.

For more information, please refer to *Antibody Purification Handbook* (see "Ordering information").

Scale-up

The easiest way to scale up is to go from a 1 mL HiTrap column to a 5 mL column. Alternatively, scale-up of small scale purifications can be done by coupling the columns in series (back pressure will increase).

Further scale-up can be done with bulk packages using nProtein A Sepharose Fast Flow, rProtein A Sepharose Fast Flow, or Protein G Sepharose Fast Flow.

Storage

Recommended storage conditions for HiTrap rProtein A FF, HiTrap Protein A HP, and HiTrap Protein G HP is in 20% ethanol at 2°C to 8°C.

Table 2. Relative binding strengths of protein A and protein G

Species	Subclass	Protein A binding	Protein G binding
Human	IgA	varible	–
	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		+/–	++

* Purified using HiTrap IgM Purification HP columns
† Purified using HiTrap IgY Purification HP columns
++++ = strong binding
++ = medium binding
+/– = weak or no binding

Column volume	20 mL
Column dimensions	1.6 × 10 cm
Recommended operating flow rate[1]	2 to 10 mL/min (60 to 300 cm/h)
Maximum operating flow rate[1]	10 mL/min (300 cm/h)
Maximum pressure over the packed bed during operation	0.15 MPa (1.5 bar, 22 psi)
Column hardware pressure limit	0.5 MPa (5 bar, 73 psi)

[1] At room temperature using water

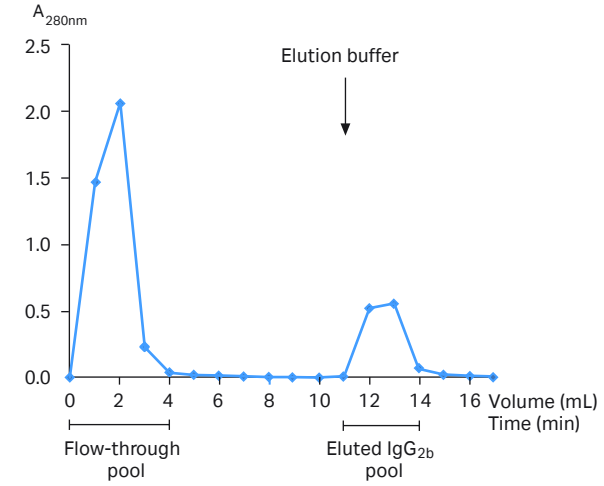
HiTrap rProtein A FF

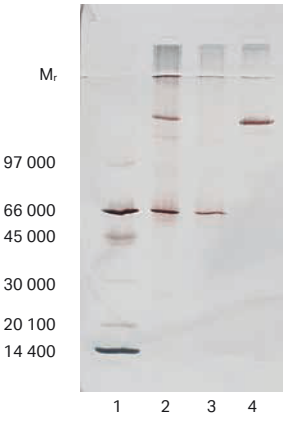
Purification of monoclonal mouse IgG_{2b} from ascites

Mouse IgG_{2b} was purified on HiTrap rProtein A FF 1 mL column operated with a syringe. The eluted pool contained 1 mg IgG_{2b}.

The silver-stained SDS-PAGE confirmed that the eluted antibody was over 95% pure (Fig 3).

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: LMW markers
Lane 2: Starting material, mouse ascites, diluted 1:10
Lane 3: Flow-through pool
Lane 4: Eluted IgG_{2b}

Fig 3. Purification of mouse IgG_{2b} from ascites on HiTrap rProtein A FF 1 mL column using a syringe.

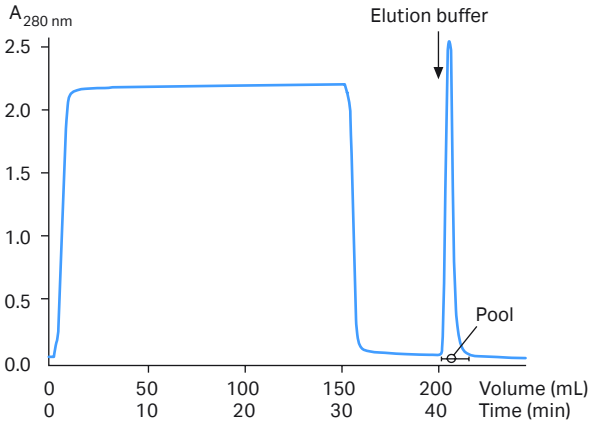
Purification of monoclonal mouse IgG1 from cell culture supernatant

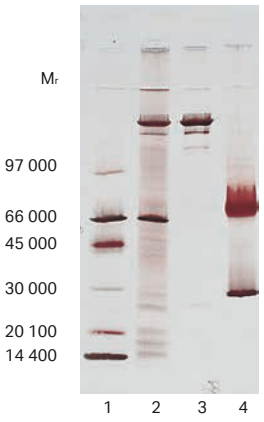
Mouse IgG_1 was purified from 150 mL cell culture supernatant on HiTrap rProtein A FF 5 mL column.

The eluted pool contained 28 mg IgG_1.

The eluted IgG_1 was over 95 % pure according to SDS-PAGE with silver-staining (Fig 4).

Sample:	150 mL of cell culture supernatant containing IgG, 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 markers
Lane 2: Starting material, cell culture supernatant
Lane 3: Eluted IgG_1 pool, diluted 1:5, non-reduced condition
Lane 4: Eluted IgG_1 pool, diluted 1:5, reduced condition

Fig 4. Purification of mouse IgG_1 from cell culture supernatant on HiTrap rProtein A FF 5 mL column.

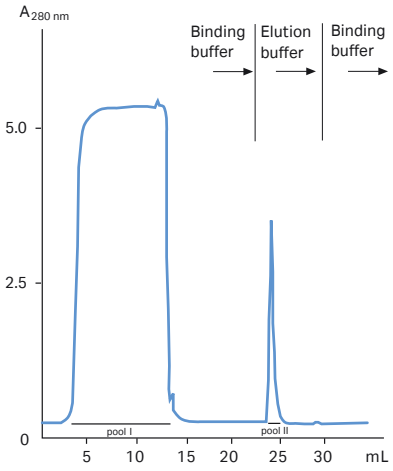
HiTrap Protein A HP and HiTrap Protein G HP

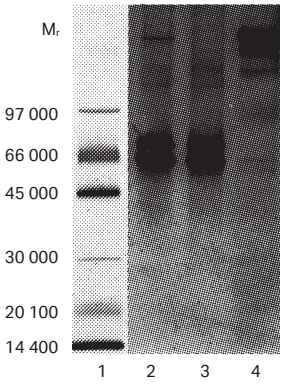
Purification of monoclonal mouse IgG_{2b}

Mouse IgG_{2b} was purified on HiTrap rProtein A FF 1 mL column operated with a syringe. The eluted pool contained 1 mg IgG_{2b}.

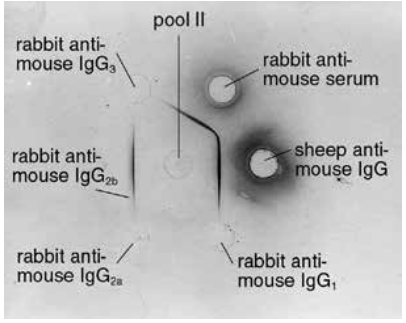
The silver-stained SDS-PAGE confirmed that the eluted antibody was over 95% pure, (Fig 3).

Sample:	10 mL mouse IgG_{2b} hybridoma cell culture fluid
Column:	HiTrap Protein A HP 1 mL
Binding buffer:	0.02 M sodium phosphate, pH 7.0
Elution buffer:	0.1 M citric acid-NaOH, pH 3.0
Chromatographic procedure:	2 mL binding buffer, 10 mL sample, 10 mL binding buffer, 5 mL elution buffer, 5 mL binding buffer. The eluted fractions were neutralized with 1 M Tris-HCl, pH 9.0
Electrophoresis:	SDS-PAGE, PhastSystem, PhastGel Gradient 10–15, 1 µL sample, silver stained
Immunodiffusion:	1% Agarose A in 0.75 M Tris, 0.25 M 5,5-diethylbarbituric acid, *5 mM Ca-lactate, 0.02% sodium azide, pH 8.6*





Lane 1: LMW markers
Lane 2: Mouse hybridoma cell culture fluid, non-reduced, diluted 1:10
Lane 3: Pool I, unbound material, non-reduced, diluted 1:10
Lane 4: Pool II, purified mouse IgG_{2b}, non-reduced, diluted 1:10



Immunodiffusion

Fig 5. Purification of monoclonal mouse IgG_{2b} on HiTrap Protein A HP.

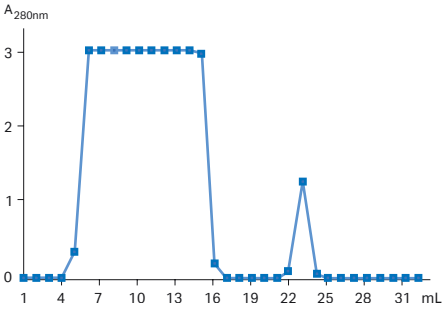
Purification of mouse monoclonal IgG_1 from cell culture supernatant

Mouse monoclonal cell supernatant IgG_1, anti-transferrin, was purified on HiTrap Protein G HP using syringe operation and pump operation.

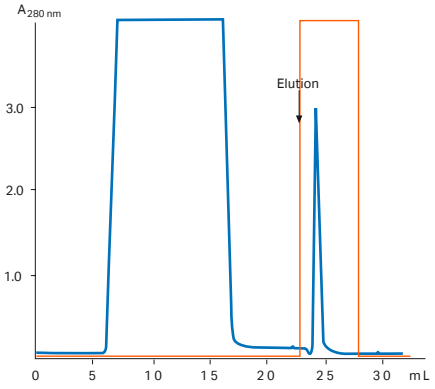
The purity was checked with SDS-PAGE, (Fig 6).

Sample:	10 mL mouse monoclonal cell supernatant, IgG_1, anti-transferrin
Column:	HiTrap Protein G HP 1 mL
Binding buffer:	0.02 M sodium phosphate, pH 7.0
Elution buffer:	0.1 M glycine-HCl, pH 2.7
Electrophoresis:	SDS-PAGE, PhastSystem, PhastGel Gradient 10–15, 1 µL sample, silver stained

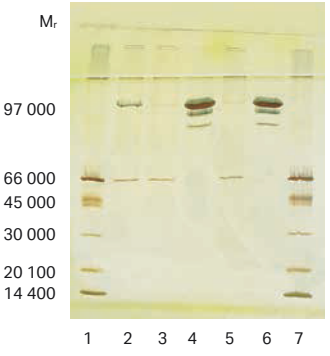
(A)



(B)



(C)



Lane 1 and 7: LMW markers
Lane 2: Crude cell culture supernatant, mouse IgG_1, diluted 1:10
Lane 3: Flow through, using peristaltic pump, diluted 1:10
Lane 4: Eluted mouse IgG_1, using a peristaltic pump
Lane 5: Flow through, using a syringe, diluted 1:10
Lane 6: Eluted mouse IgG_1, using a syringe

Fig 6. Purification of mouse monoclonal IgG_1 from cell culture supernatant (A) syringe operation; (B) pump operation; (C) SDS-PAGE on PhastSystem using PhastGel 10-15, non-reduced condition, and silver staining.

Purification of monoclonal mouse Ig_{G1} from hybridoma cell culture

Mouse IgG_1 hybridoma cell culture fluid was purified on HiTrap Protein G HP. The purity was checked with SDS-PAGE (Fig 7).

Tables 3 and 4 list physio-chemical data for human and mouse immunoglobulins.

Sample: 12 mL mouse IgG_1 hybridoma cell culture fluid
Column: HiTrap Protein G HP 1 mL
Flow rate: 1.0 mL/min
Binding buffer: 0.02 M sodium phosphate, pH 7.0
Elution buffer: 0.1 M glycine-HCl, pH 2.7
Procedure: 5 mL binding buffer, 12 mL sample, 10 mL binding buffer 6 mL elution buffer, 7 mL binding buffer. The eluted fractions were neutralized with 1 M Tris-HCl, pH 9.0
Electrophoresis: SDS-PAGE, PhastSystem, PhastGel Gradient 10–15, 1 µL sample, silver stained
Immunodiffusion: 1% agarose A in 0.75 M Tris, 0.25 M 5,5-diethylbarbituric acid, 5 mM Ca-lactate, 0.02% sodium azide, pH 8.6

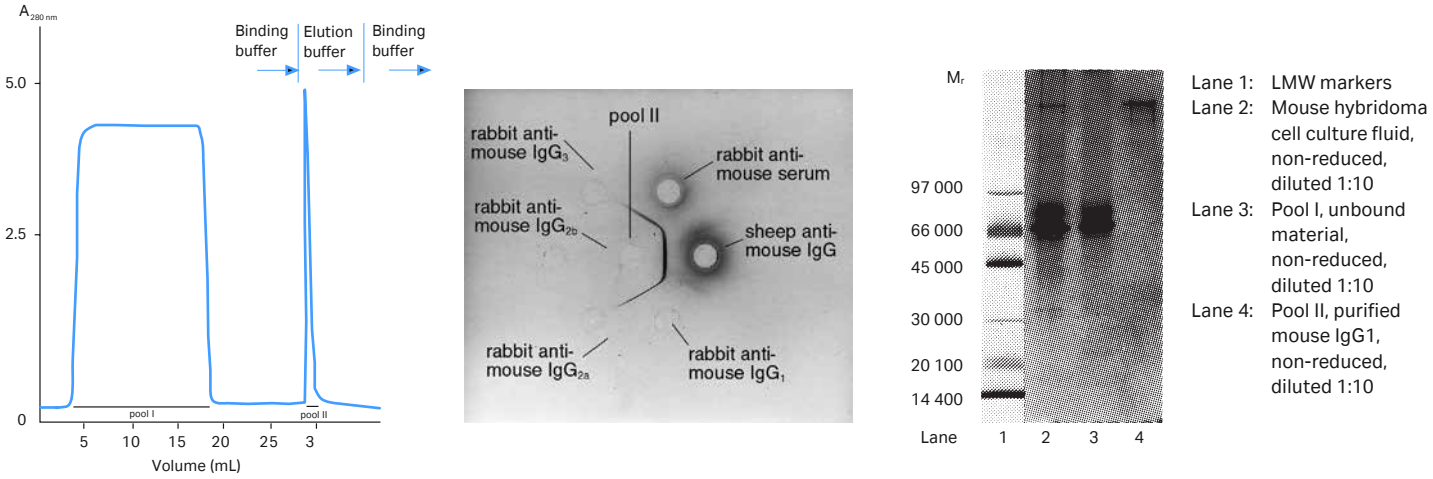


Fig 7. Purification of monoclonal mouse IgG_1 on HiTrap Protein G HP, 1 mL.

Table 3. Physio-chemical properties of human immunoglobulins

Immunoglobulin	Heavy chain	Light chain	Sedimentation coefficient	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 4. Physio-chemical properties of mouse immunoglobulins

Immunoglobulin	Heavy chain	Light chain	Sedimentation coefficient	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	–

Ordering information

Product	Quantity	Code number
HiTrap Protein A HP	5 × 1 mL	17040201
HiTrap Protein A HP	2 × 1 mL	17040203
HiTrap Protein A HP	1 × 1 mL	29048576
HiTrap Protein A HP	1 × 5 mL	17040301
HiTrap Protein A HP	5 × 5 mL	17040303
HiTrap Protein G HP	5 × 1 mL	17040401
HiTrap Protein G HP	2 × 1 mL	17040403
HiTrap Protein G HP	1 × 1 mL	29048581
HiTrap Protein G HP	1 × 5 mL	17040501
HiTrap Protein G HP	5 × 5 mL	17040503
HiTrap rProtein A FF	5 × 1 mL	17507901
HiTrap rProtein A FF	2 × 1 mL	17507902
HiTrap rProtein A FF	1 × 5 mL	17508001
HiTrap rProtein A FF	5 × 5 mL	17508002

Related products

HiTrap Desalting	5 × 5 mL	17140801
HiTrap Desalting	1 × 5 mL	29048684
HiTrap Desalting	100 × 5 mL*	11000329
HiPrep™ 26/10 Desalting	1 × 53 mL	17508701
HiPrep 26/10 Desalting	4 × 53 mL	17508702
MAbTrap™ Kit	1 kit	17112801
nProtein A Sepharose 4 Fast Flow	5 mL	17528001
nProtein A Sepharose 4 Fast Flow	25 mL	17528004
rProtein A Sepharose 4 Fast Flow	5 mL	17127901
rProtein A Sepharose 4 Fast Flow	25 mL	17127902
Protein G Sepharose 4 Fast Flow	5 mL	17061801
Protein G Sepharose 4 Fast Flow	25 mL	17061802
HiTrap MabSelect SuRe™	5 × 1 mL	11003493
HiTrap MabSelect SuRe	1 × 1 mL	29049104
HiTrap MabSelect SuRe	1 × 5 mL	11003494
HiTrap MabSelect SuRe	5 × 5 mL	11003495
HiTrap MabSelect PrismA	1 × 1 mL	17549851
HiTrap MabSelect PrismA	5 × 1 mL	17549852
HiTrap MabSelect PrismA	1 × 5 mL	17549853
HiTrap MabSelect PrismA	5 × 5 mL	17549854
MabSelect PrismA	25 mL	17549801
MabSelect PrismA	200 mL	17549802
MabSelect SuRe	25 mL	17543801
MabSelect Xtra™	25 mL	17526907
MabSelect™	25 mL	17519901

* Pack size available by special order.

Accessories	Quantity	Code number
1/16" male/luer female*	2	18111251
Tubing connector flangeless/M6 female	2	18100368
Tubing connector flangeless/M6 male	2	18101798
Union 1/16" female/M6 male	6	18111257
Union M6 female/1/16" male	5	18385801
Union luerlock female/M6 female	2	18102712
HiTrap/HiPrep, 1/16" male connector for ÄKTA systems	8	28401081
Stop plug female, 1/16"†	5	11000464
Fingertight stop plug, 1/16"‡	5	11000355

Related literature

Purification handbook	18103746
Affinity chromatography handbook, principle and methods	18102229
Affinity chromatography columns and media product profile	18112186

* One connector included in each HiTrap package
† Two, five, or seven stop plugs female included in HiTrap packages depending on products
‡ One fingertight stop plug is connected to the top of each HiTrap column at delivery

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CY15715-25Sep20-DF

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