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

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 easy to use HiTrap column and the various affinity media.

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
- Easy to use with syringe, pump or chromatography system such as ÄKTAdesign™ or FPLC™ System

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 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 and protein G for IgG depends on the source species of the particular immunoglobulin.

Figure 1. HiTrap rProtein A FF, HiTrap Protein A HP and HiTrap Protein G HP are designed for puification of monoclonal and polyclonal IgG.

The capacity depends also upon several other factors such as flow rate during sample application, and sample concentration.

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, see Table 2 for more details.

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 ÄKTAdesign systems.

Media characteristics

rProtein A Sepharose Fast Flow

rProtein A and protein A share similar specificity for the Fc-region of IgG, but recombinant protein A (rProtein A) 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 om human IgG contamination.

Recombinant *(E. coli)* protein A ligand is immobilized to Sepharose™ Fast Flow, a robust highly cross-linked agarose with spherical 90 µm beads.

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 nature of the agarose base provides a hydrophilic and chemically favourable environment for coupling, while the highly cross-linked structure of the 34 µm spherical beads 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 depends also upon several other factors, such as flow rate during sample application and sample concentration. As a reference, the binding capacity for human IgG is approximately 20 mg IgG/ml medium for HiTrap Protein A HP, approximately 25 mg IgG/ml medium for HiTrap Protein G HP and approximately 50 mg/ml medium for HiTrap rProtein A FF.

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

Operation

All HiTrap columns are quick and easy to use. Instructions and several different 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, for example when linear gradients are required or when 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 can not be opened or repacked.

Column volume	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
Ligand concentration (Approx.)	6 mg rProtein A/ml medium (HiTrap rProtein A FF) 3 mg protein A /ml medium (HiTrap Protein A HP) 2 mg protein G/ml medium (HiTrap Protein G HP)
Binding capacity (Approx.)	50 mg human IgG/ml medium (HiTrap rProtein A FF) 20 mg human IgG (HiTrap Protein A HP) 25 mg human IgG (HiTrap Protein G HP)
Dynamic binding capacities* (HiTrap rProtein A FF)	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 (HiTrap rProtein A FF) 34 µm (HiTrap Protein A HP and HiTrap Protein G HP)
Bead structure	Highly cross-linked spherical agarose
Recommended flow rate	1 and 5 ml/min for 1 and 5 ml column respectively
Maximum flow rate†	4 and 20 ml/min for 1 and 5 ml column respectively
Maximum back pressure	0.3 MPa, 3 bar
pH stability‡	
Long term	3–10 (HiTrap rProtein A FF) 3–9 (HiTrap Protein A HP and HiTrap Protein G HP)
Short term	2–11 (HiTrap rProtein A FF) 2–10 (HiTrap Protein A HP) 2–9 (HiTrap Protein G HP)
Storage	+4 to +8 °C in 20 % ethanol

* 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

† 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 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 cleaning.

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

Figure 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 in 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 +4 to +8 °C.

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

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

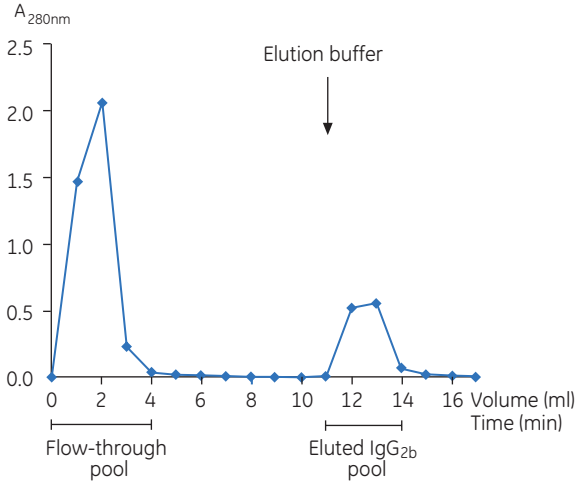
HiTrap rProtein A FF

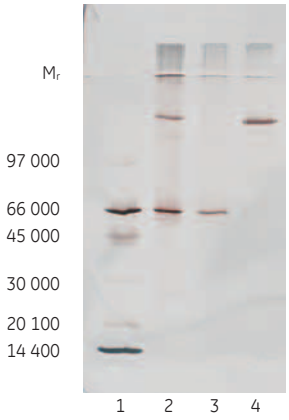
Purification of monoclonal mouse IgG_{2b} from ascites

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:	approx. 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}

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

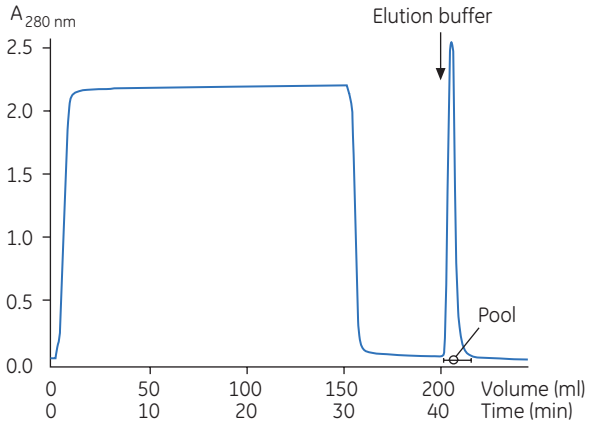
Purification of monoclonal mouse IgG_1 from cell culture supernatant

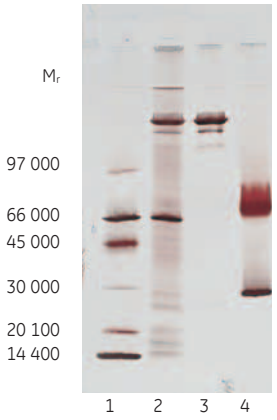
Mouse IgG_1 was purified from 150 ml cell culture supernatant on HiTrap rProtein A FF 5 ml column.

The 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

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

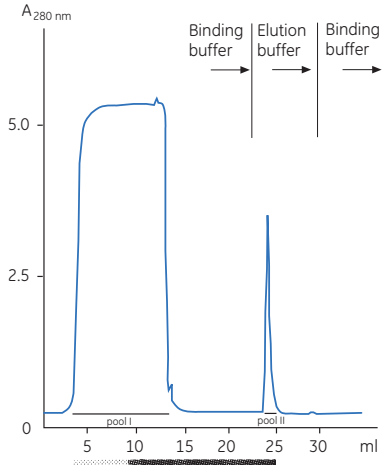
HiTrap Protein A HP and HiTrap Protein G HP

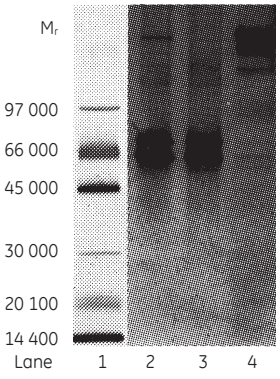
Purification of monoclonal mouse IgG_{2b}

Mouse IgG_{2b} from hybridoma cell culture fluid was purified on HiTrap Protein A HP.

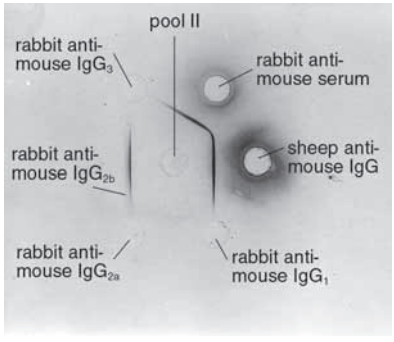
The purity was checked with SDS-PAGE, (Fig 5).

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

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

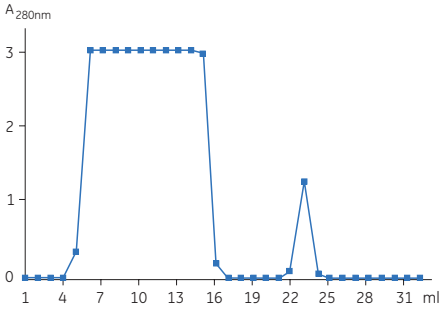
Purification of mouse monoclonal IgG_1 from cell culture supernatant

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

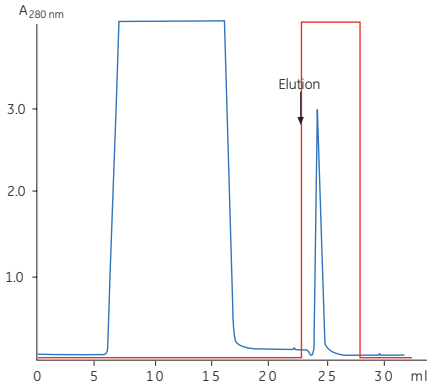
The purity was checked with SDS-PAGE, (Fig 6).

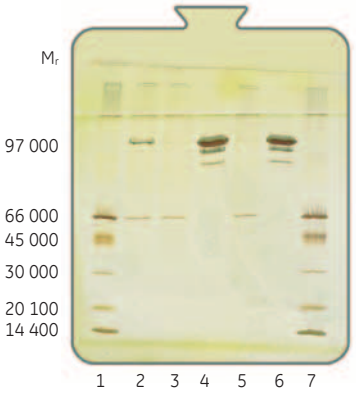
Sample:	10 ml mouse monoclonal cell supernatant, IgG_1, anti-transferrin
Column:	HiTrap Protein G HP 1 ml
Binding buffer:	0.02 M sodium phosphate, pH 7.0
Elution buffer:	0.1 M glycine-HCl, pH 2.7
Electrophoresis:	SDS-PAGE, PhastSystem, PhastGel Gradient 10–15, 1 µl sample, silver stained

A) Syringe operation, approximately 60 drops/min



B) Pump operation, flow rate 2 ml/min





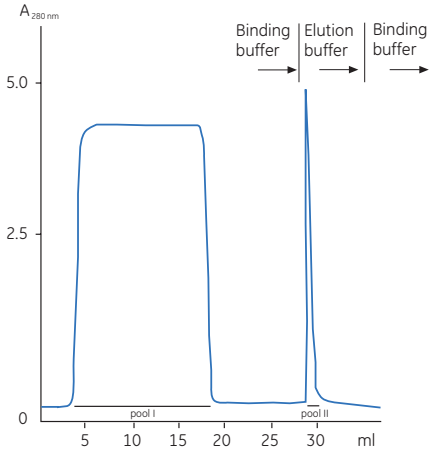
Lane 1 and 7: LMW markers
Lane 2: Crude cell culture supernatant, mouse IgG_1, diluted 1:10
Lane 3: Flow through, using peristaltic pump, diluted 1:10
Lane 4: Eluted mouse IgG_1, using a peristaltic pump
Lane 5: Flow through, using a syringe, diluted 1:10
Lane 6: Eluted mouse IgG_1, using a syringe

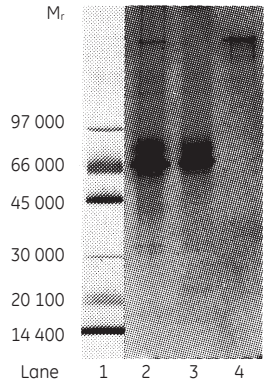
Figure 6. Purification of mouse monoclonal IgG_1 from cell culture supernatant. **A.** with syringe operation. **B.** with pump operation SDS-PAGE on PhastSystem using PhastGel 10-15, non-reduced condition, and silver staining.

Purification of monoclonal mouse IgG_1 from hybridoma cell culture

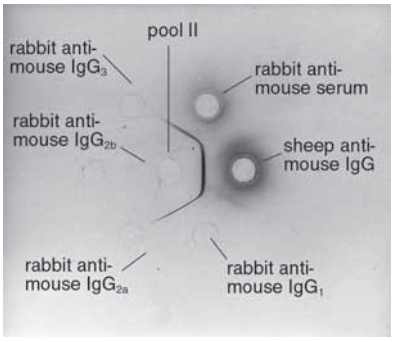
Mouse IgG_1 hybridoma cell culture fluid was purified on HiTrap Protein G HP. The purity was checked with SDS-PAGE, (Fig 7).

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
Chromatographic 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





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_1, non-reduced, diluted 1:10



Immunodiffusion

Figure 7. Purification of monoclonal mouse IgG_1 on HiTrap Protein G HP, 1 ml.

Table 3 and 4 lists physio-chemical data for human and mouse 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 3. Physio-chemical properties of human 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	–

Table 4. Physio-chemical properties of mouse immunoglobulins.

Ordering information

Product	Quantity	Code No.
HiTrap Protein A HP	5 × 1 ml	17-0402-01
HiTrap Protein A HP	2 × 1 ml	17-0402-03
HiTrap Protein A HP	1 × 5 ml	17-0403-01
HiTrap Protein A HP	5 × 5 ml	17-0403-03
HiTrap Protein G HP	5 × 1 ml	17-0404-01
HiTrap Protein G HP	2 × 1 ml	17-0404-03
HiTrap Protein G HP	1 × 5 m	17-0405-01
HiTrap Protein G HP	5 × 5 ml	17-0405-03
HiTrap rProtein A FF	5 × 1 ml	17-5079-01
HiTrap rProtein A FF	2 × 1 ml	17-5079-02
HiTrap rProtein A FF	1 × 5 ml	17-5080-01
HiTrap rProtein A FF	5 × 5 ml	17-5080-02

Related products	Quantity	Code No.
HiTrap Desalting	5 × 5 ml	17-1408-01
HiTrap Desalting	100 × 5 ml*	11-0003-29
HiPrep™ 26/10 Desalting	1 × 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 × 53 ml	17-5087-02
MAbTrap™ Kit	1 kit	17-1128-01
nProtein A Sepharose 4 Fast Flow	5 ml	17-5280-01
nProtein A Sepharose 4 Fast Flow	25 ml	17-5280-04
rProtein A Sepharose 4 Fast Flow	5 ml	17-1279-01
rProtein A Sepharose 4 Fast Flow	25 ml	17-1279-02
Protein G Sepharose 4 Fast Flow	5 ml	17-0618-01
Protein G Sepharose 4 Fast Flow	25 ml	17-0618-02

* Pack size available by special order.

Related products	Quantity	Code No.
HiTrap MabSelect SuRe™	5 × 1 ml	11-0034-93
HiTrap MabSelect SuRe	1 × 5 ml	11-0034-94
HiTrap MabSelect SuRe	5 × 5 ml	11-0034-95
MabSelect SuRe	25 ml	17-5438-01
MabSelect Xtra™	25 ml	17-5269-07
MabSelect™	25 ml	17-5199-01

Accessories	Quantity	Code No.
1/16" male/Luer female	2	18-1112-51
Union luerlock female/M6 female	2	18-1027-12
Union 1/16" female/ M6 male	6	18-1112-57
Tubing connector flangeless/M6 male	2	18-1017-98
Tubing connector flangeless/M6 female	2	18-1003-68
Union M6 female/1/16" Male	5	18-3858-01
Stop plug female, 1/16"	5	11-0004-64
Fingertight stop plug, 1/16"	5	11-0003-55

Related literature	Code No.
Antibody Purification Handbook	18-1037-46
Affinity Chromatography Handbook, Principle and Methods	18-1022-29
Affinity Chromatography Columns and Media Product Profile	18-1121-86
Convenient Protein Purification, HiTrap Column Guide	18-1129-81

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