HiTrap™ MabSelect SuRe™ HiTrap MabSelect™ HiTrap MabSelect Xtra™

HiTrap MabSelect SuRe (Superior Resistance), HiTrap MabSelect, and HiTrap MabSelect Xtra (Fig 1) are members of the HiTrap family of prepacked columns for purification of monoclonal antibodies (MAbs).

Key performance characteristics of HiTrap MabSelect products include:

- Increased binding capacity compared with other protein A media
- High purity in one step
- Convenient, prepacked 1 ml and 5 ml columns
- Excellent for process development and screening of purification conditions
- Simple operation with a syringe, a pump, an ÄKTA™ system, or other chromatography systems.

The protein A ligand used in all three MabSelect media is produced by validated fermentation and downstream processes. The entire production process is free of components of mammalian origin. The resulting highly purified ligand is immobilized to the agarose matrix through a chemically stable thio-ether linkage.

HiTrap MabSelect SuRe

MabSelect SuRe is composed of a rigid, high-flow agarose matrix and alkali-stabilized protein A-derived ligand that allows the usage of up to 0.5 M NaOH for cleaning-in-place (CIP). This ligand provides greater stability than conventional protein A-based media in the alkaline conditions used in CIP protocols. This is important when the same column is used for purification of different antibodies, as performing

Fig 1. HiTrap MabSelect SuRe, HiTrap MabSelect, and HiTrap MabSelect Xtra 1 and 5 ml for purification of monoclonal antibodies with optimized binding capacities at high flow rates.

CIP will prevent cross-contamination between the different purifications. The enhanced alkali stability of MabSelect SuRe also improves purification economy; cleaning can be performed with cost-effective reagents such as 0.1 to 0.5 M sodium hydroxide.

The MabSelect SuRe ligand was developed by protein engineering of one of the IgG-binding domains of protein A. Amino acids particularly sensitive to alkali were identified and substituted with more stable ones. The final construct is a tetramer of the engineered domain with a C-terminal cysteine, which enables single-point attachment to the matrix.

The combination of low ligand leakage and high dynamic binding capacity together with the high-flow matrix makes MabSelect SuRe well suited for the purification of MAbs when scaling up. Characteristics of HiTrap MabSelect SuRe are listed in Table 1.

HiTrap MabSelect

MabSelect features a highly cross-linked agarose, produced using a manufacturing process that gives a very rigid matrix that is optimal for high-throughput affinity chromatography of IgG. The matrix of MabSelect allows at least five-times higher flow rates to be used in process scale compared with conventional cross-linked agarose of similar porosity. The epoxy-based coupling chemistry ensures low ligand leakage. The high capacity, low ligand leakage, and specially developed base matrix make MabSelect ideal for purification of monoclonal antibodies at process scale. See Table 2 for a summary of characteristics.

HiTrap MabSelect Xtra

MabSelect Xtra has been developed to meet the demands of ever-increasing levels of expression in monoclonal antibody feedstocks. MabSelect Xtra uses the same recombinant protein A ligand as MabSelect, but has a smaller particle size and greater porosity, which ensures increased dynamic binding capacity. The medium provides a lower overall production cost due to the possibility of processing concentrated feedstocks in fewer batches. The characteristics of HiTrap MabSelect Xtra are listed in Table 3.

Column characteristics

HiTrap columns are made of biocompatible polypropylene and they have porous top and bottom frits. Columns are delivered with a stopper on the inlet and a snap-off end on the outlet. Note that HiTrap columns cannot be opened or repacked.

Low ligand leakage

All three MabSelect media exhibit only a low level of ligand leakage during elution. Leakage is affected by chromatographic running conditions and the composition of the sample. For detailed information, please refer to the instructions for each media.

Table 1. Characteristics of HiTrap MabSelect SuRe

Medium	MabSelect SuRe
Matrix	Rigid, highly cross-linked agarose
Average particle size	85 µm
Ligand	Alkali-tolerant, protein A-derived *(E. coli)*
Coupling chemistry	Epoxy
Dynamic binding capacity[1]	Approx. 30 mg human IgG/ml medium
Column volumes	1 ml or 5 ml
Column dimensions	0.7 × 2.5 cm (1 ml) 1.6 × 2.5 cm (5 ml)
Recommended flow rate	1 and 5 ml/min for 1 and 5 ml column respectively
Maximum flow rates	4 and 20 ml/min for 1 and 5 ml column respectively
Column hardware pressure limit	5 bar (0.5 MPa, 70 psi)
Chemical stability[2]	Stable in all aqueous buffers commonly used in protein A chromatography: 10 mM HCl (pH 2), 10 mM NaOH (pH 12), 0.1 M sodium citrate/HCl (pH 3), 6 M guanidine HCl, 20% ethanol, 2% benzyl alcohol
pH stability[3]	
Working[4]	3 to 12
Cleaning[5]	2 to 13
Storage	4°C to 8°C in 20% ethanol

[1] Determined at 10% breakthrough by frontal analysis at 250 cm/h in a column with a bed height of 10 cm. Residence time is 2.4 min. Residence time is equal to bed height (cm) divided by nominal flow velocity (cm/h) during sample loading. Flow velocity is equal to flow rate (ml/h) divided by column cross-sectional area (cm²).

Note: The dynamic binding capacity may decrease for columns with a bed height of 2.5 cm at the recommended flow rate (1 ml/min for 1 ml column and 5 ml/min for 5 ml column), as residence time may be too short for optimal binding.

[2] No significant change in chromatographic performance after 1 week storage, or 100 cycles normal use at room temperature, which corresponds to a total contact time of 16.7 h (10 mM NaOH).

[3] pH below 3 is sometimes required to elute strongly bound IgG species. However, protein ligands may hydrolyze at very low pH

[4] Refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance

[5] Refers to the pH interval for regeneration

Table 2. Characteristics of HiTrap MabSelect

Medium	MabSelect
Matrix	Rigid, highly cross-linked agarose
Average particle size	85 µm
Ligand	Recombinant protein A *(E. coli)*
Coupling chemistry	Epoxy
Dynamic binding capacity[1]	Approx. 30 mg human IgG/ml medium
Column volumes	1 ml or 5 ml
Column dimensions	0.7 × 2.5 cm (1 ml) 1.6 × 2.5 cm (5 ml)
Recommended flow rate	1 and 5 ml/min for 1 and 5 ml columns, respectively
Maximum flow rates	4 and 20 ml/min for 1 and 5 ml columns, respectively
Column hardware pressure limit	5 bar (0.5 MPa, 70 psi)
Chemical stability[2]	Stable in all aqueous buffers commonly used in protein A chromatography: 10 mM HCl (pH 2), 10 mM NaOH (pH 12), 0.1 M sodium citrate/HCl (pH 3), 6 M guanidine HCl, 20% ethanol, 2% benzyl alcohol
pH stability[3]	
Working[4]	3 to 10
Cleaning[5]	2 to 12
Storage	4°C to 8°C in 20% ethanol

[1] Determined at 10% breakthrough by frontal analysis at 500 cm/h in a column with a bed height of 20 cm, i.e. residence time is 2.4 min. Residence time is equal to bed height (cm) divided by flow velocity (cm/h) during sample loading. Flow velocity (cm/h) is equal to flow rate (ml/h) divided by column cross-sectional area (cm²).

Note: The dynamic binding capacity may decrease for columns with bed height of 2.5 cm at the recommended flow rate (1 ml/min for 1 ml column or 5 ml/min for 5 ml column), due to too short residence time for optimal binding.

[2] No significant change in chromatographic performance after 1 week storage, or 100 cycles normal use at room temperature, which corresponds to a total contact time of 16.7 h (10 mM NaOH).

[3] pH below 3 is sometimes required to elute strongly bound IgG species. However, protein ligands may hydrolyze at very low pH

[4] Refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance

[5] Refers to the pH interval for regeneration

High dynamic binding capacities

The recombinant protein A in MabSelect media has been engineered to include a C-terminal cysteine. The coupling conditions are controlled to favor a thioether coupling providing single point attachment of the protein A. The oriented coupling also enhances the binding of IgG.

The dynamic binding capacity (DBC) of MabSelect media is affected by the flow rate, or residence time. The lower the flow rate (or the higher the residence time), the higher the DBC (Figs 2 and 3). A residence time of 2.4 min yields the specified binding capacities for the media, as stated in Tables 1 to 3. The dynamic binding capacity may decrease for columns with a bed height of 2.5 cm at the recommended flow rates (1 ml/min for 1 ml column or 5 ml/min for 5 ml column) because the residence time will be too short to ensure optimal binding.

Table 3. Characteristics of HiTrap MabSelect Xtra

Medium	MabSelect Xtra
Matrix	Rigid, highly cross-linked agarose
Average particle size	75 µm
Ligand	Recombinant protein A *(E. coli)*
Coupling chemistry	Epoxy
Dynamic binding capacity[1]	Approx. 40 mg human IgG/ml medium
Column volumes	1 ml or 5 ml
Column dimensions	0.7 × 2.5 cm (1 ml) 1.6 × 2.5 cm (5 ml)
Recommended flow rate	1 and 5 ml/min for 1 and 5 ml columns, respectively
Maximum flow rates	4 and 20 ml/min for 1 and 5 ml columns, respectively
Column hardware pressure limit	5 bar (0.5 MPa, 70 psi)
Chemical stability[2]	Stable in all aqueous buffers commonly used in protein A chromatography: 10 mM HCl (pH 2), 10 mM NaOH (pH 12), 0.1 M sodium citrate/HCl (pH 3), 6 M guanidine HCl, 20% ethanol, 2% benzyl alcohol
pH stability[3]	
Working[4]	3 to 10
Cleaning[5]	2 to 12
Storage	4°C to 8°C in 20% ethanol

[1] Determined at 10% breakthrough by frontal analysis at 250 cm/h in a column with a bed height of 10 cm, i.e. residence time is 2.4 min. Residence time is equal to bed height (cm) divided by flow velocity (cm/h) during sample loading. Flow velocity (cm/h) is equal to flow rate (ml/h) divided by column cross-sectional area (cm²).

Note: The dynamic binding capacity may decrease for columns with bed height of 2.5 cm at the recommended flow rate (1 ml/min for 1 ml column or 5 ml/min for 5 ml column), due to too short residence time for optimal binding.

[2] No significant change in chromatographic performance after 1 week storage, or 100 cycles normal use at room temperature, which corresponds to a total contact time of 16.7 h (10 mM NaOH).

[3] pH below 3 is sometimes required to elute strongly bound IgG species. However, protein ligands may hydrolyze at very low pH

[4] Refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance

[5] Refers to the pH interval for regeneration

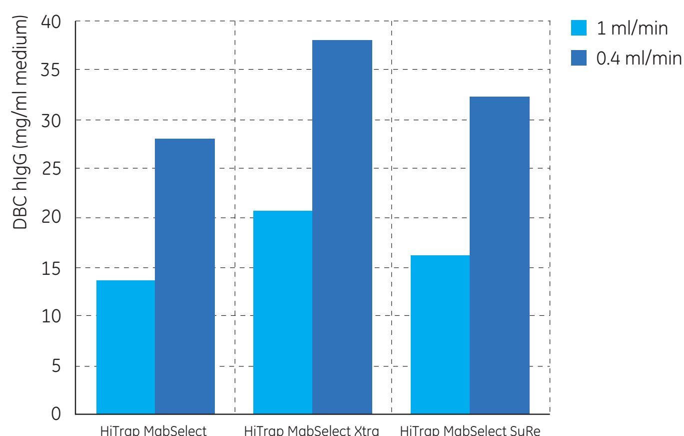


Fig 2. Comparison of dynamic binding capacities (DBC) of HiTrap MabSelect, HiTrap MabSelect Xtra, and HiTrap MabSelect SuRe 1 ml columns at two different flow rates. Sample: 1 mg/ml human IgG (hIgG) Gammanorm™ (Octapharma); Binding buffer: 0.02 M phosphate, 0.15 M NaCl, pH 7.4; Elution buffer: 0.1 M sodium citrate, pH 3.0. Samples were run on an ÄKTAexplorer 10 system.

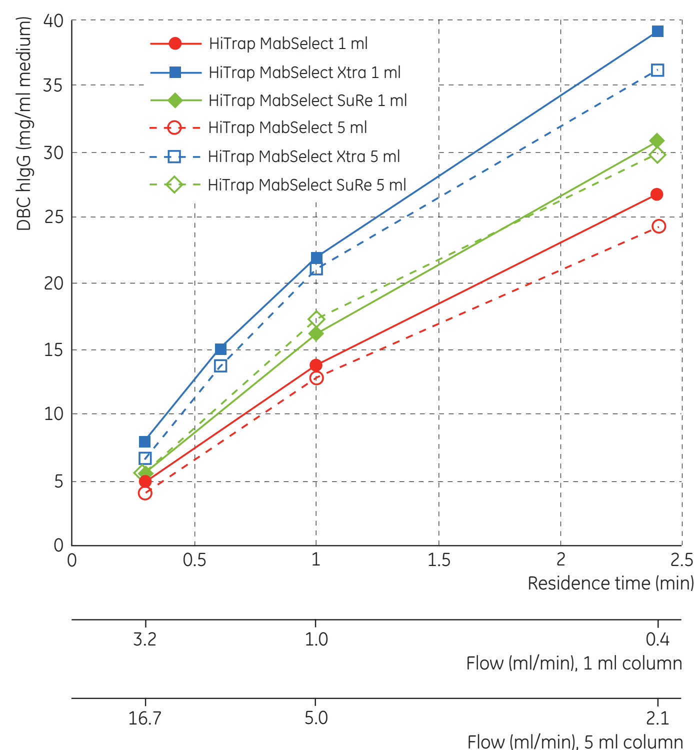


Fig 3. Dynamic binding capacity (DBC) as a function of residence time and flow rates for HiTrap MabSelect, HiTrap MabSelect Xtra, and HiTrap MabSelect SuRe 1 ml and 5 ml columns. Sample: 1 mg/ml human IgG (hIgG) Gammanorm (Octapharma); Binding buffer: 0.02 M phosphate, 0.15 M NaCl, pH 7.4; Elution buffer: 0.1 M sodium citrate, pH 3.0. Samples were run on either an ÄKTAexplorer 10 or ÄKTAexplorer 100 system.

Cleaning-in-place using HiTrap MabSelect SuRe

CIP is an essential step in the purification of MAbs. The main drawback with using sodium hydroxide for CIP of conventional protein A-based media is the sensitivity of native and recombinant Protein A to alkaline conditions. MabSelect SuRe, however, retains dynamic binding capacity after repeated CIP cycles with 0.5 M NaOH.

Approximately 85% to 90% of the initial dynamic binding capacity of MabSelect SuRe is retained after numerous CIP cycles with 0.5 M sodium hydroxide (Fig 4).

Rigorous CIP with sodium hydroxide reduces the risk of both contamination from host cell proteins and microbial growth in the prepacked column, as well as carry-over in the purified antibody.

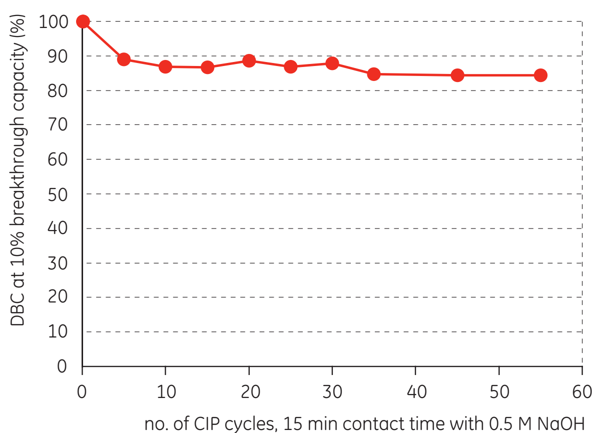


Fig 4. Dynamic binding capacity for HiTrap MabSelect SuRe 1 ml for polyclonal human IgG after more than 50 CIP cycles with 0.5 M NaOH.

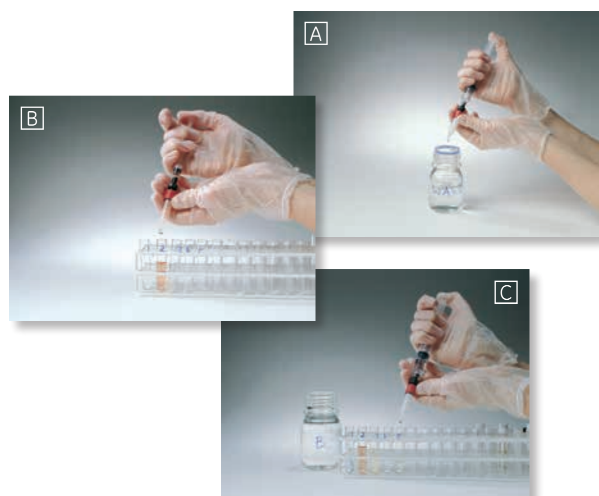


Fig 5. Using HiTrap MabSelect products 1 ml with a syringe. (A) Prepare buffers and sample. Remove the stop-plug from the top of the column and snap-off the end. Wash and equilibrate. (B) Load the sample and begin collecting fractions. (C) Wash and elute, continue collecting fractions.

Operation and method optimization

HiTrap MabSelect, HiTrap MabSelect SuRe, and HiTrap MabSelect Xtra 1 ml and 5 ml columns bring added time-savings, convenience, and reliability to the purification of antibodies. The columns can be easily used with a pump or a chromatography system such as an ÄKTA chromatography system or FPLC system. The columns are also simple to operate with a syringe and the supplied Luer connector (Fig 5). Note that ÄKTA systems include preset method templates for these columns, which further enhances operation, especially reproducibility. In addition, multiple HiTrap columns can be easily connected in series for increased purification capacity.

Table 4. 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		+/–	++

* Purify using HiTrap IgM Purification HP columns.
† Purify using HiTrap IgY Purification HP columns.

++++ = strong binding
++ = medium binding
– = weak or no binding

The primary aim of method optimization is to establish the conditions that will bind the highest amount of target molecule, in the shortest time, and with the highest product recovery. The degree to which IgG binds to protein A varies with respect to both origin and antibody subclass (Table 4). Typically, the clarified feedstock is loaded onto the column directly.

After optimizing the antibody purification at laboratory scale, the process can be scaled up by keeping the residence time constant in order to maintain capacity. This can be achieved by increasing the column diameter, and keeping the mobile phase velocity and sample-to-bed volume ratio constant.

Cleaning

It is important to clean a column between samples when the same column is used for the purification of different antibodies. Proper cleaning will prevent cross-contamination between the different samples. For detailed information, please refer to the instructions for each media.

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.

The different MabSelect media are is also available in bulk packages for further scale-up.

Storage

Recommended storage conditions for HiTrap MabSelect SuRe, HiTrap MabSelect, and HiTrap MabSelect Xtra are in 20% ethanol at 4°C to 8°C.

Applications

Single-step purification

Different samples of human IgG were purified on HiTrap MabSelect SuRe 1 ml (Fig 6), and HiTrap MabSelect Xtra 1 ml (Fig 7). Both media yielded highly pure antibody, as shown by SDS-PAGE, in a single purification step.

Automatic two-step purification

HiTrap MabSelect, HiTrap MabSelect SuRe, and HiTrap MabSelect Xtra are easy to use with ÄKTAxpress MAb, which offers automated, multistep, high-throughput purification of monoclonal antibodies. Examples of two-step purifications (affinity followed by gel filtration) for each column are shown in Figures 8 to 10.

Acknowledgement

Samples of mouse IgG_{2a} and IgG_1 were supplied by kind courtesy of Phadia AB, Uppsala, Sweden. Human IgG_1 was supplied in collaboration with Polymun Scientific, Vienna, Austria.

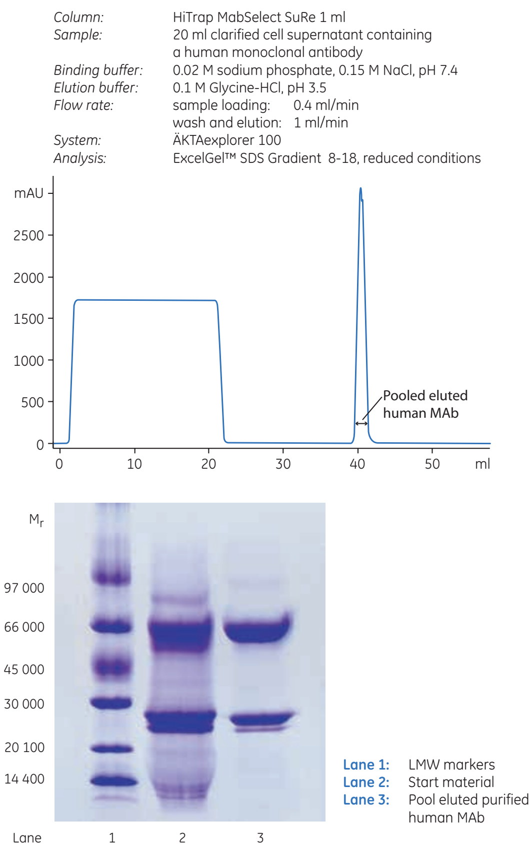


Fig 6. Purification of a human monoclonal antibody (MAb) on HiTrap MabSelect SuRe 1 ml on ÄKTAexplorer 100. For quick neutralization of eluted antibodies, 1 M Tris, pH 9, was added to the tubes.

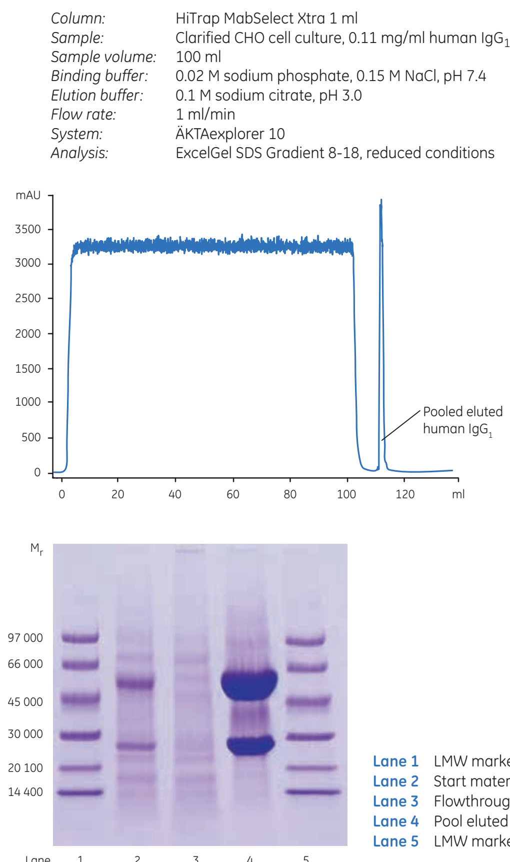
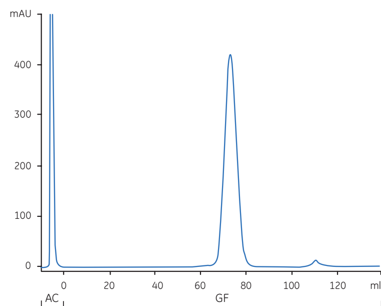


Fig 7. Purification of human IgG_1 on HiTrap MabSelect Xtra 1 ml on ÄKTAexplorer 10. For quick neutralization of eluted antibodies, 1 M Tris, pH 9, was added to the tubes.

Affinity column:	HiTrap MabSelect 1 ml
Gel filtration column:	HiLoad™ 16/60 Superdex™ 200 pg
Sample:	Filtered mouse myeloma cell culture, 165 mg/l IgG_{2a}
Sample volume:	75 ml
Binding buffer (affinity):	0.02 M sodium phosphate, 0.15 M NaCl, pH 7.4
Elution buffer (affinity):	0.1 M sodium citrate, pH 3.0
Buffer (gel filtration):	0.15 M NaCl
Flow rate:	
affinity:	1 ml/min
gel filtration:	1.5 ml/min
System:	ÄKTAxpress MAb
Analysis:	ExcelGel SDS Gradient 8-18, reduced conditions



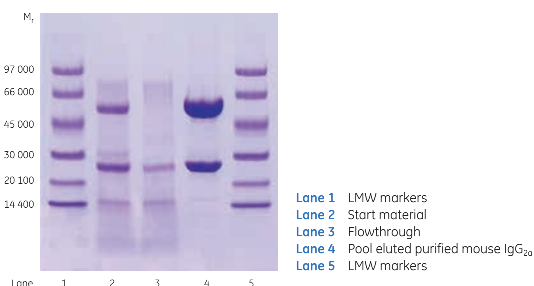
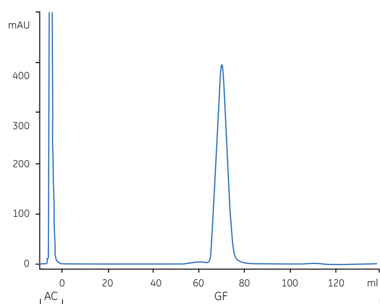


Fig 8. Purification of IgG_{2a} using HiTrap MabSelect 1 ml in an automated two-step purification on ÄKTAxpress MAb. Yield of purified antibody was 9.2 mg at a concentration of 0.75 mg/ml.

Affinity column:	HiTrap MabSelect Xtra 1 ml
Gel filtration column:	HiLoad 16/60 Superdex 200 pg
Sample:	Filtered mouse myeloma cell culture, 54 mg/l IgG_1
Sample volume:	150 ml
Binding buffer (affinity):	0.1 M sodium phosphate, 2.5 M NaCl, pH 7.0
Elution buffer (affinity):	0.1 M sodium citrate, pH 3.0
Buffer (gel filtration):	0.15 M NaCl
Flow rate:	
affinity:	1 ml/min
gel filtration:	1.5 ml/min
System:	ÄKTAxpress MAb
Analysis:	ExcelGel SDS Gradient 8-18, reduced conditions



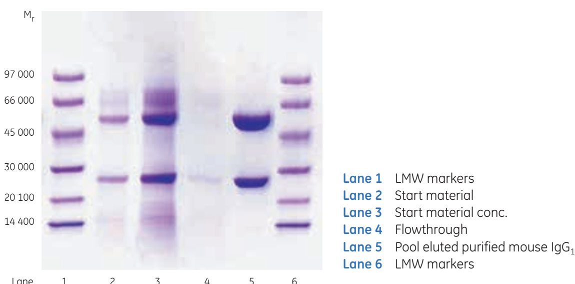


Fig 9. Purification of IgG_1 using HiTrap MabSelect Xtra 1 ml in an automated two-step purification on ÄKTAxpress MAb. Yield of purified antibody was 7.6 mg at a concentration of 0.72 mg/ml.

Affinity column:	HiTrap MabSelect SuRe 1 ml
Gel filtration column:	HiLoad 16/60 Superdex 200 pg
Sample:	Filtered mouse myeloma cell culture, 54 mg/l IgG_1
Sample volume:	200 ml
Binding buffer (affinity):	0.1 M sodium phosphate, 2.5 M NaCl, pH 7.0
Elution buffer (affinity):	0.1 M sodium citrate, pH 3.0
Buffer (gel filtration):	0.15 M NaCl
Flow rate:	
affinity:	1 ml/min
gel filtration:	1.5 ml/min
System:	ÄKTAxpress MAb
Analysis:	ExcelGel SDS Gradient 8-18, reduced conditions

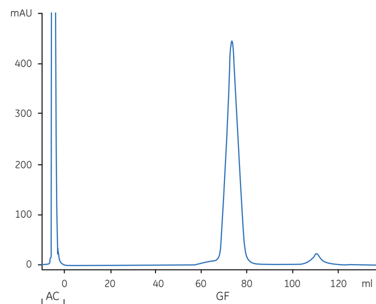


Fig 10. Purification of IgG_1 using HiTrap MabSelect SuRe 1 ml in an automated two-step purification on ÄKTAxpress MAb. Yield of purified antibody was 9.6 mg at a concentration of 0.78 mg/ml.

Ordering Information

Product	Quantity	Code number
HiTrap MabSelect SuRe	1 × 1 ml	29-0491-04
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
HiTrap MabSelect	5 × 1 ml	28-4082-53
HiTrap MabSelect	1 × 5 ml	28-4082-55
HiTrap MabSelect	5 × 5 ml	28-4082-56
HiTrap MabSelect Xtra	5 × 1 ml	28-4082-58
HiTrap MabSelect Xtra	1 × 5 ml	28-4082-60
HiTrap MabSelect Xtra	5 × 5 ml	28-4082-61

Related products	Quantity	Code number
MabSelect SuRe	25 ml	17-5438-01
MabSelect SuRe	200 ml*	17-5438-02
MabSelect	25 ml	17-5199-01
MabSelect	200 ml*	17-5199-02
MabSelect Xtra	25 ml	17-5269-07
MabSelect Xtra	200 ml*	17-5269-02
HiTrap Desalting	1 × 5 ml	29-0486-84
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

* Larger pack sizes are available
† Pack size available by special order

Accessories	Quantity	Code number
1/16" male/luer female*	2	18-1112-51
Tubing connector flangeless/M6 female	2	18-1003-68
Tubing connector flangeless/M6 male	2	18-1017-98
Union 1/16" female/M6 male	6	18-1112-57
Union M6 female /1/16" male	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"†	5	11-0004-64
Fingertight stop plug, 1/16"‡	5	11-0003-55

* 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.

Related literature	Code number
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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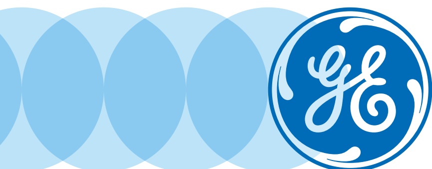

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