

# HiTrap MabSelect SuRe

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# HiTrap MabSelect Xtra

## AFFINITY CHROMATOGRAPHY

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 resins
- 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 resins 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 resins in the alkaline conditions used in CIP protocols. This is important when the same column is used for purification of different antibodies, as performing CIP will prevent cross-contamination between the different purifications. The enhanced alkali stability of



**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.

MabSelect SuRe also improves purification economy; cleaning can be performed with cost-effective reagents such as 0.1 to 0.5 M NaOH.

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 resin 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.

**Table 1.** Characteristics of HiTrap MabSelect SuRe

Resin	MabSelect Sure
Matrix	Highly cross-linked agarose, spherical
Particle size, $d_{50V}$ <sup>1</sup>	~ 85 $\mu\text{m}$
Ligand	Alkali-tolerant, protein A-derived ( <i>E. coli</i> )
Coupling chemistry	Epoxy
Dynamic binding capacity, $Q_{B10}$ <sup>2</sup>	~ 35 mg hlgG/mL resin
Chemical stability	Stable to commonly used aqueous buffers, 10 mM NaOH (pH 12), 0.1 M sodium citrate/HCl (pH 3), 6 M guanidine HCl, 20% ethanol, 2% benzyl alcohol
pH stability, operational <sup>3</sup>	3 to 12
pH stability, CIP <sup>4</sup>	3.0 to 13.7
Column volumes	1 mL or 5 mL
Column dimensions	0.7 $\times$ 2.5 cm (1 mL) 1.6 $\times$ 2.5 cm (5 mL)
Recommended operating flow rate <sup>5</sup>	0.5 mL/min for 1 mL and 2.5 mL/min for 5 mL column
Maximum operating flow rate <sup>5</sup>	4 mL/min for 1 mL and 20 mL/min for 5 mL column
Column hardware pressure limit	5 bar (0.5 MPa, 70 psi)
Storage	20% ethanol, 2°C to 8°C

<sup>1</sup> Median particle size of the cumulative volume distribution.

<sup>2</sup> Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 500 cm/h in a XK 16/20 column at 20 cm bed height (2.4 min residence time) for human IgG in 0.020 M  $\text{NaH}_2\text{PO}_4$ , pH 7.4.

<sup>3</sup> pH range where resin can be operated without significant change in function.

<sup>4</sup> pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

<sup>5</sup> At room temperature using buffers with the same viscosity as water.

## 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 resins 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 resin.

## High dynamic binding capacities

The recombinant protein A in MabSelect resins 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.

**Table 2.** Characteristics of HiTrap MabSelect

Resin	MabSelect
Matrix	Highly cross-linked agarose, spherical
Particle size, $d_{50V}$ <sup>1</sup>	~ 85 $\mu\text{m}$
Ligand	Recombinant protein A ( <i>E. coli</i> )
Coupling chemistry	Epoxy
Dynamic binding capacity, $Q_{B10}$ <sup>2</sup>	~ 30 mg hlgG/mL resin
Chemical stability	Stable to commonly used aqueous buffers, 10 mM NaOH (pH 12), 0.1 M sodium citrate/HCl (pH 3), 6M guanidine HCl, 8M urea, 20% ethanol, 2% benzyl alcohol
pH stability, operational <sup>3</sup>	3 to 10 <sup>4</sup>
pH stability, CIP <sup>5</sup>	3.0 to 12.4 <sup>6</sup>
Column volumes	1 mL or 5 mL
Column dimensions	0.7 $\times$ 2.5 cm (1 mL) 1.6 $\times$ 2.5 cm (5 mL)
Recommended operating flow rate <sup>7</sup>	0.5 mL/min for 1 mL and 2.5 mL/min for 5 mL column
Maximum operating flow rate <sup>7</sup>	4 mL/min for 1 mL and 20 mL/min for 5 mL column
Column hardware pressure limit	5 bar (0.5 MPa, 70 psi)
Storage	20% ethanol, 2°C to 8°C

<sup>1</sup> Median particle size of the cumulative volume distribution.

<sup>2</sup> Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 500 cm/h in a XK 16/20 column at 20 cm bed height (2.4 min residence time) for human IgG in 0.020 M  $\text{NaH}_2\text{PO}_4$ , pH 7.4.

<sup>3</sup> pH range where resin can be operated without significant change in function.

<sup>4</sup> pH below 3 is sometimes required to elute strongly bound IgG species. However, protein ligands may hydrolyze at pH below 2.

<sup>5</sup> pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

<sup>6</sup> Reducing agent e.g. 100 mM 1-Thioglycerol followed by 15 mM NaOH is among the most efficient CIP for MabSelect.

<sup>7</sup> At room temperature using buffers with the same viscosity as water.

The dynamic binding capacity (DBC) of MabSelect resins 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 resins, as stated in Tables 1 to 3. The DBC might 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.

Resin	MabSelect Xtra
Matrix	Highly cross-linked agarose, spherical
Particle size, $d_{50V}$ <sup>1</sup>	~ 75 $\mu$ m
Ligand	Recombinant protein A ( <i>E. coli</i> )
Coupling chemistry	Epoxy
Dynamic binding capacity, $Q_{B10}$ <sup>2</sup>	~ 40 mg hIgG/mL resin
Chemical stability	Stable to commonly used aqueous buffers, 10 mM NaOH (pH 12), 0.1 M sodium citrate/HCl (pH 3), 6M guanidine HCl, 20% ethanol, 2% benzyl alcohol
pH stability, operational <sup>3</sup>	3 to 10 <sup>4</sup>
pH stability, CIP <sup>5</sup>	3.0 to 12.4 <sup>6</sup>
Column volumes	1 mL or 5 mL
Column dimensions	0.7 × 2.5 cm (1 mL) 1.6 × 2.5 cm (5 mL)
Recommended operating flow rate <sup>7</sup>	0.5 mL/min for 1 mL and 2.5 mL/min for 5 mL column
Maximum operating flow rate <sup>7</sup>	4 mL/min for 1 mL and 20 mL/min for 5 mL column
Column hardware pressure limit	5 bar (0.5 MPa, 70 psi)
Storage	20% ethanol, 2 °C to 8 °C

<sup>1</sup> Median particle size of the cumulative volume distribution.

<sup>2</sup> Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 500 cm/h in a XK 16/20 column at 20 cm bed height (2.4 min residence time) for human IgG in 0.020 M  $\text{NaH}_2\text{PO}_4$ , pH 7.4

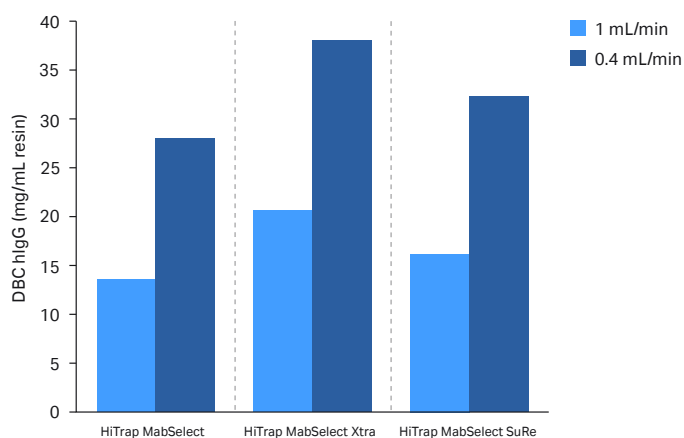
<sup>3</sup> pH range where resin can be operated without significant change in function.

<sup>4</sup> pH below 3 is sometimes required to elute strongly bound IgG species. However, protein ligands may hydrolyze at pH below 2.

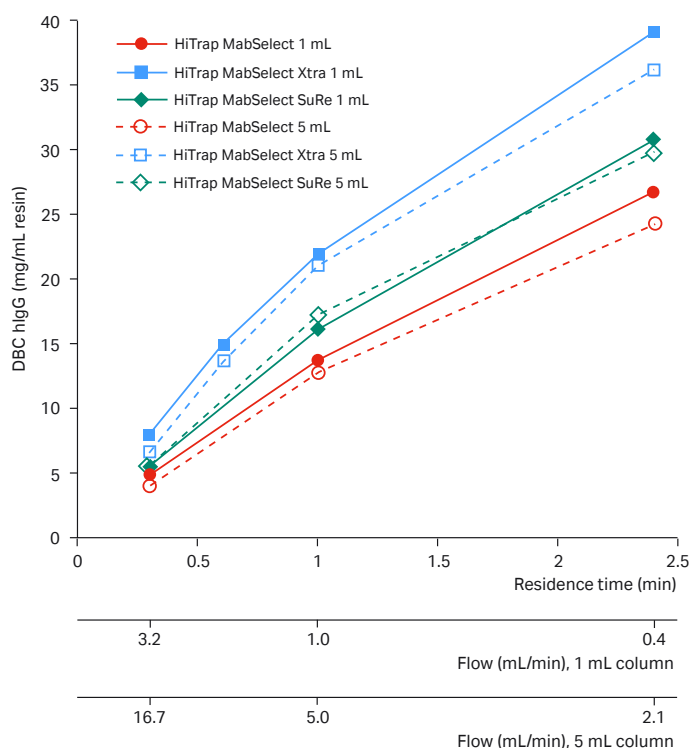
<sup>5</sup> pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

<sup>6</sup> Reducing agent e.g. 100 mM 1-Thioglycerol followed by 15 mM NaOH is among the most efficient CIP for MabSelect.

<sup>7</sup> At room temperature using buffers with the same viscosity as water.



**Fig 2.** Comparison of 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 (hlgG) 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.



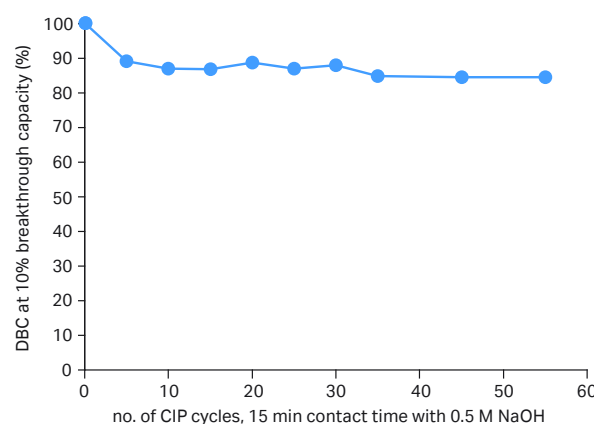
**Fig 3.** Dynamic binding capacity (DBC) as a function of residence time and flow rates for HiTrap MabSelect, HiTrap MabSelect Xtra, and HiTrap MabSelect SuRe 1 mL and 5 mL columns. Sample: 1 mg/mL human IgG (hlgG) Gammanorm (Octapharma); Binding buffer: 0.02 M phosphate, 0.15 M NaCl, pH 7.4; Elution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

## CIP using HiTrap MabSelect SuRe

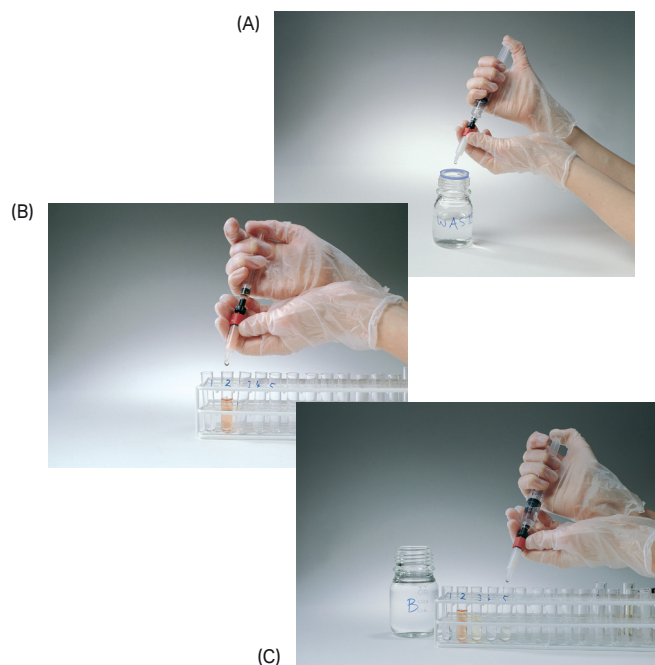
CIP is an essential step in the purification of mAbs. The main drawback with using sodium hydroxide for CIP of conventional protein A-based resins is the sensitivity of native and recombinant protein A to alkaline conditions. MabSelect SuRe, however, retains dynamic binding capacity after repeated CIP 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.** DBC 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	variable	–
	IgD	–	–
	IgE	–	–
	IgG <sub>1</sub>	++++	++++
	IgG <sub>2</sub>	++++	++++
	IgG <sub>3</sub>	–	++++
	IgG <sub>4</sub>	++++	++++
	IgM*	variable	–
Avian egg yolk	IgY†	–	–
Cow		++	++++
Dog		++	+
Goat		–	++
Guinea pig	IgG <sub>1</sub>	++++	++
	IgG <sub>2</sub>	++++	++
Hamster		+	++
Horse		++	++++
Koala		–	+
Llama		–	+
Monkey (rhesus)		++++	++++
Mouse	IgG <sub>1</sub>	+	++++
	IgG <sub>2a</sub>	++++	++++
	IgG <sub>2b</sub>	+++	+++
	IgG <sub>3</sub>	++	+++
	IgM*	variable	–
Pig		+++	+++
Rabbit	no distinction	++++	+++
Rat	IgG <sub>1</sub>	–	+
	IgG <sub>2a</sub>	–	++++
	IgG <sub>2b</sub>	–	++
	IgG <sub>3</sub>	+	++
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 resin.

## 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 resins are 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 2°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 resins yielded highly pure antibody, as shown by SDS-PAGE, in a single purification step.

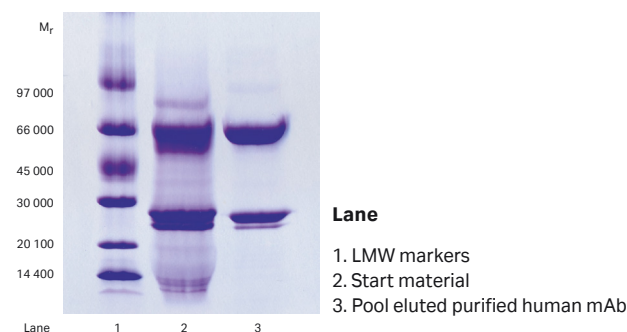
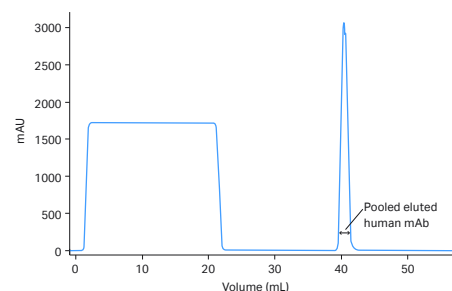
## Automatic two-step purification

HiTrap MabSelect, HiTrap MabSelect SuRe, and HiTrap MabSelect Xtra are easy to use with chromatography systems that offers automated, multistep, high-throughput purification of monoclonal antibodies. Examples of two-step purifications (affinity followed by size exclusion chromatography) for each column are shown in Figures 8 to 10.

## Acknowledgement

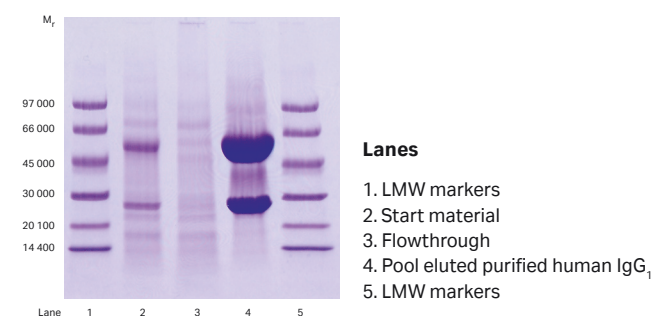
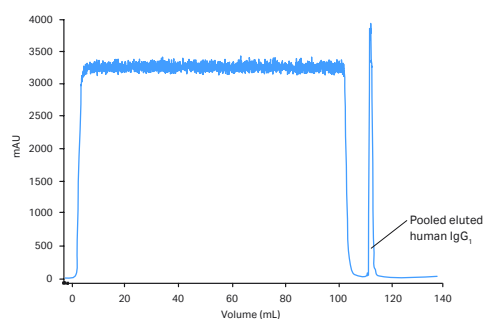
Samples of mouse IgG<sub>2a</sub> and IgG<sub>1</sub> were supplied by kind courtesy of Phadia AB, Uppsala, Sweden. Human IgG<sub>1</sub> was supplied in collaboration with Polymun Scientific, Vienna, Austria.

**Column:** HiTrap MabSelect SuRe 1 mL  
**Sample:** 20 mL clarified cell supernatant containing a human monoclonal antibody  
**Binding buffer:** 0.02 M sodium phosphate, 0.15 M NaCl, pH 7.4  
**Elution buffer:** 0.1 M Glycine-HCl, pH 3.5  
**Flow rate:** sample loading: 0.4 mL/min  
 wash and elution: 1 mL/min  
**System:** ÄKTAexplorer 100  
**Analysis:** ExcelGel™ SDS Gradient 8 to 18, reduced conditions



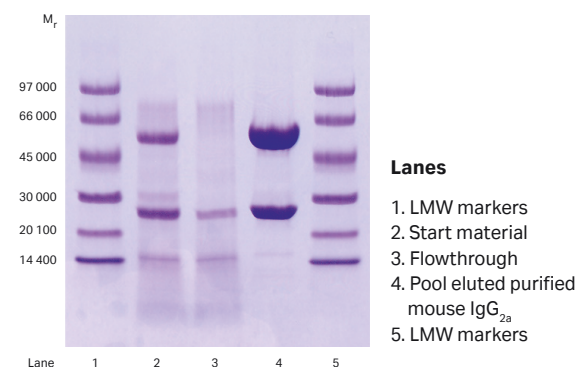
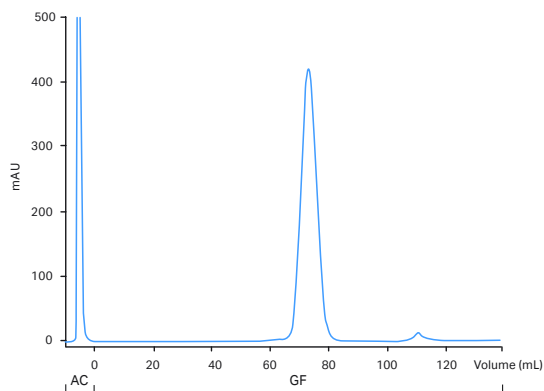
**Fig 6.** Purification of a human monoclonal antibody (mAb) on HiTrap MabSelect SuRe 1 mL on ÄKTAexplorer 100. For quick neutralization of eluted antibodies, 1 M Tris, pH 9, was added to the tubes.

**Column:** HiTrap MabSelect Xtra 1 mL  
**Sample:** Clarified CHO cell culture, 0.11 mg/ml human IgG<sub>1</sub>, 100 mL  
**Sample volume:** 100 mL  
**Binding buffer:** 0.02 M sodium phosphate, 0.15 M NaCl, pH 7.4  
**Elution buffer:** 0.1 M sodium citrate, pH 3.0  
**Flow rate:** 1 mL/min  
**System:** ÄKTAexplorer 10  
**Analysis:** ExcelGel SDS Gradient 8-18, reduced conditions



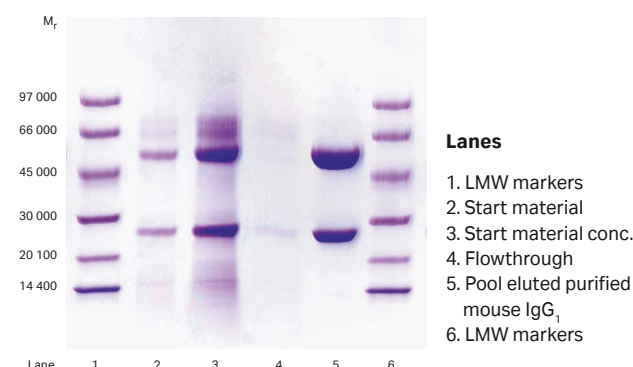
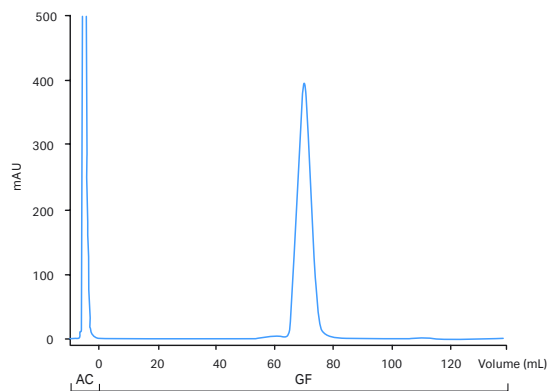
**Fig 7.** Purification of human IgG<sub>1</sub> on HiTrap MabSelect Xtra 1 mL on ÄKTAexplorer 10. For quick neutralization of eluted antibodies, 1 M Tris, pH 9, was added to the tubes.

**Affinity column:** HiTrap MabSelect 1 mL  
**Size exclusion chromatography column:** HiLoad™ 16/60 Superdex™ 200 pg  
**Sample:** Filtered mouse myeloma cell culture, 165 mg/l IgG<sub>2a</sub>  
**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 (size exclusion):** 0.15 M NaCl  
**Flow rate:**  
     **Affinity:** 1 mL/min  
     **Size exclusion:** 1.5 mL/min  
**System:** ÄKTApurify mAb  
**Analysis:** ExcelGel SDS Gradient 8 to 18, reduced conditions



**Fig 8.** Purification of IgG<sub>2a</sub> using HiTrap MabSelect 1 mL in an automated two-step purification on ÄKTApurify mAb. Yield of purified antibody was 9.2 mg at a concentration of 0.75 mg/mL.

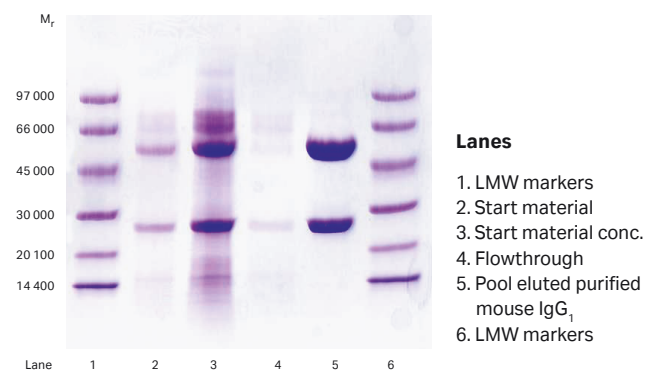
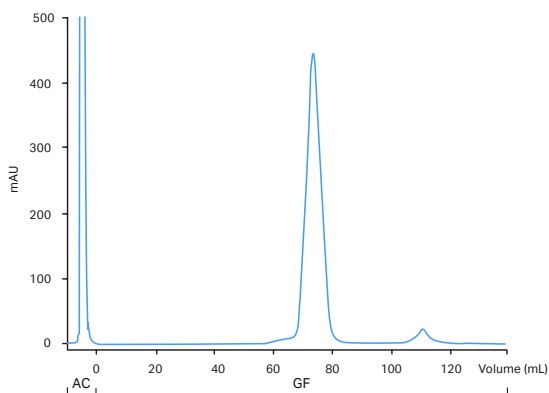
**Affinity column:** HiTrap MabSelect Xtra 1 mL  
**Size exclusion chromatography column:** HiLoad 16/60 Superdex 200 pg  
**Sample:** Filtered mouse myeloma cell culture, 54 mg/l IgG<sub>1</sub>  
**Sample volume:** 150 mL  
**Binding buffer (affinity):** 0.1 M sodium phosphate, 2.5 M NaCl, pH 7.0  
**Elution buffer (affinity):** 0.1 M sodium citrate, pH 3.0  
**Buffer (size exclusion):** 0.15 M NaCl  
**Flow rate:**  
     **Affinity:** 1 mL/min  
     **Size exclusion:** 1.5 mL/min  
**System:** ÄKTApurify mAb  
**Analysis:** ExcelGel SDS Gradient 8 to 18, reduced conditions



**Fig 9.** Purification of IgG<sub>1</sub> using HiTrap MabSelect Xtra 1 mL in an automated two-step purification on ÄKTApurify mAb. Yield of purified antibody was 7.6 mg at a concentration of 0.72 mg/mL.



**Affinity column:** HiTrap MabSelect SuRe 1 mL  
**Size exclusion chromatography column:** HiLoad 16/60 Superdex 200 pg  
**Sample:** Filtered mouse myeloma cell culture, 54 mg/IgG<sub>1</sub>, 200 mL  
**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 (size exclusion):** 0.15 M NaCl  
**Flow rate:**  
**Affinity:** 1 mL/min  
**Size exclusion:** 1.5 mL/min  
**System:** ÄKTAexpress mAb  
**Analysis:** ExcelGel SDS Gradient 8 to 18, reduced conditions



**Fig 10.** Purification of IgG<sub>1</sub> using HiTrap MabSelect SuRe 1 mL in an automated two-step purification on ÄKTAexpress mAb. Yield of purified antibody was 9.6 mg at a concentration of 0.78 mg/mL.

## Ordering Information

Product	Quantity	Product code
HiTrap MabSelect SuRe	1 × 1 mL	29049104
	5 × 1 mL	11003493
	1 × 5 mL	11003494
	5 × 5 mL	11003495
HiTrap MabSelect	5 × 1 mL	28408253
	1 × 5 mL	28408255
	5 × 5 mL	28408256
HiTrap MabSelect Xtra	5 × 1 mL	28408258
	1 × 5 mL	28408260
	5 × 5 mL	28408261

### Related products

MabSelect SuRe	25 mL	17543801
	200 mL*	17543802
HiTrap MabSelect Prisma	1 × 1 mL	17549851
	5 × 1 mL	17549852
	1 × 5 mL	17549853
	5 × 5 mL	17549854
MabSelect	25 mL	17519901
	200 mL*	17519902
MabSelect Xtra	25 mL	17526907
	200 mL*	17526902
HiTrap Desalting	1 × 5 mL	29048684
	5 × 5 mL	17140801
	100 × 5 mL†	11000329
HiPrep™ 26/10 Desalting	1 × 53 mL	17508701
	4 × 53 mL	17508702

\* Larger pack sizes are available

† Pack size available by special order

### Accessories

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 design	8	28401081
Stop plug female, 1/16"†	5	11000464
Fingertight stop plug, 1/16"‡	5	11000355

\* 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

Antibody Purification Handbook	18103746
Affinity Chromatography Handbook, Principle and Methods	18102229
Affinity Chromatography Columns and Media Product Profile	18112186

## **[cytiva.com/protein-purification](https://www.cytiva.com/protein-purification)**

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