Prepacked chromatography columns

HiTrap™ MabSelect™ PrismA
HiScreen™ MabSelect PrismA

MabSelect PrismA is a next-generation protein A affinity chromatography resin that offers significantly enhanced alkaline stability and capacity for improved performance in antibody purifications. The HiTrap MabSelect PrismA and HiScreen MabSelect PrismA columns are prepacked and ready-to-use, allowing for fast and reproducible purification of antibodies in a convenient format (Fig 1). The columns can be used for both preparative purifications in research as well as process development and optimization.

Key performance characteristics include:

- High dynamic binding capacity compared with other protein A resins for improved antibody recovery.
- Excellent alkaline stability for efficient cleaning between runs using 0.5–1.0 M NaOH.
- Formats well-suited for preparative purifications as well as for screening and optimization of purification conditions.

Product overview

Nearly all commercially approved antibody manufacturing processes utilize protein A capture as the initial step in a downstream purification process. Protein A capture is also widely used in research applications, as the technique allows for quick and convenient purification of antibodies. For research purposes, one single protein A step is often sufficient, as a purity of above 90% is usually obtained after the affinity step.

MabSelect PrismA, a next-generation protein A resin

MabSelect PrismA builds on the proven track record of the MabSelect resin family. In comparison with its predecessors, MabSelect PrismA has been improved with an optimized high-flow agarose base matrix and a genetically engineered protein A-derived ligand, allowing future demands in antibody affinity purification to be met.

Fig 1. Prepacked 1 mL and 5 mL HiTrap MabSelect PrismA and HiScreen MabSelect PrismA protein A affinity chromatography columns.

The alkaline-stability of MabSelect PrismA is important when the same column is to be used for purification of different antibodies, as efficient cleaning with sodium hydroxide at concentrations of 0.5–1.0 M will prevent cross-contamination between the different purification runs, while maintaining performance.

Convenient HiTrap and HiScreen columns for use in research and process development

HiTrap and HiScreen columns are manufactured from biocompatible polypropylene that does not interact with biomolecules. The columns can be operated with a syringe (HiTrap columns), a peristaltic pump, or a chromatography system such as the ÄKTA™ systems. The column formats are well-suited for preparative purifications as well as for screening and optimization of purification conditions when developing a new method. The column formats enable scalable experiment at relevant process flow rates. When needed, two columns can easily be connected in series to increase the bed height.

Excellent dynamic binding capacity for optimized recovery

In process development, productivity is a key parameter to optimize, and the factor influencing productivity is dynamic binding capacity (DBC). Determination of DBC under different conditions for each individual antibody will help you find optimal conditions for your process. DBC is affected by the flow rate (or residence time). The lower the flow rate (or the higher the residence time), the higher the DBC. The HiScreen column is an excellent format for method optimization and parameter screening because of the 10 cm bed height and narrow inner diameter.

Due to the enhanced properties of both the protein A ligand and the base matrix design, MabSelect PrismA offers significantly increased DBC compared with its predecessor products (Fig 2). At a residence time of 2.4 min, MabSelect PrismA offers an up to 40% increase in DBC compared with the MabSelect SuRe resin.

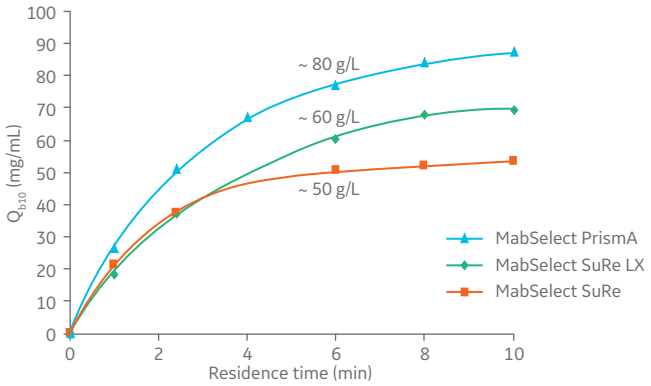


Fig 2. DBC of MabSelect PrismA compared with its predecessor MabSelect SuRe and MabSelect SuRe LX resins at 10% breakthrough (Q_{B10}), determined in HiScreen columns.

High alkaline-stability for thorough cleaning between runs

In research applications when different types of antibodies are purified on the same column, preventing cross-contamination while maintaining recovery is important. Sodium hydroxide is an efficient, low-cost, and easy-to-dispose reagent when thorough cleaning is required. Rigorous cleaning with sodium hydroxide reduces the risk of contamination from host cell proteins, microbial growth in the prepacked column, as well as carryover between purifications. However, many resins with protein-based ligands, such as protein A, are sensitive to alkaline conditions.

With the enhanced alkaline-stability of the protein A ligand, MabSelect PrismA can be cleaned with high NaOH concentrations (up to 1 M) with maintained capacity, meaning that HiTrap MabSelect PrismA and HiScreen MabSelect PrismA columns may be confidently cleaned for reuse. The resin retains more than 95% of its initial DBC after 25 cycles with 1.0 M NaOH, while only about 60% of the initial DBC of Protein A Sepharose™ High Performance, and rProtein A Sepharose Fast Flow remains after less number of CIP cycles (Fig 3). Table 1 summarize the initial DBC and the DBC at end cycle for the three resins prepacked in HiTrap columns.

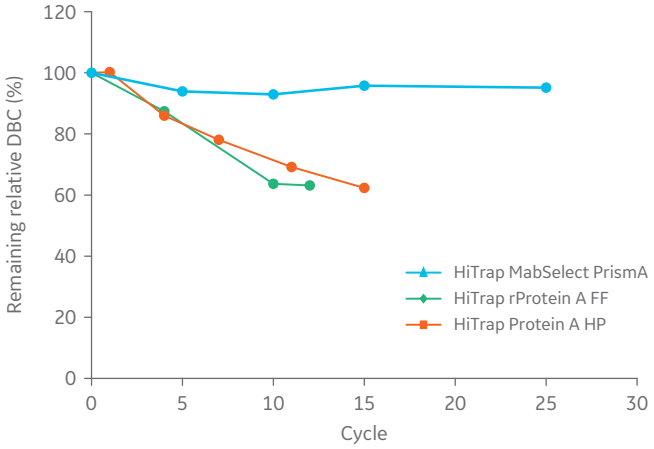


Fig 3. DBC of MabSelect PrismA, Protein A Sepharose High Performance, and rProtein A Sepharose Fast Flow for a polyclonal human IgG after multiple cycles with 1 M NaOH included in each cycle.

Table 1. Dynamic binding capacity at 10% breakthrough (Q_{B10})

	HiTrap MabSelect PrismA	HiTrap Protein A HP	HiTrap rProtein A FF
Initial DBC	41 mg/mL	25 mg/mL	28 mg/mL
DBC at end cycle	39 mg/mL	16 mg/mL	18 mg/mL
Remaining relative DBC (%)	95%	62%	63%

Recommendations for scaling up

When developing a new process, parameters such as resin selectivity and capacity as well as binding and elution conditions needs to be established. Usually screening is performed in smaller scale to save time and cost. Initially, high-throughput parallel screening of process conditions is easily performed using PreDictor™ 96-well filter plates. Scale up can be as straightforward as increasing column diameter to accommodate a larger feed volume, while keeping the bed height and the flow velocity constant. Starting from a 1 mL HiTrap column, it is easy to scale up to a 5 mL HiTrap column. Alternatively, scale up of small-scale purifications can be done by coupling the columns in series. To facilitate scale-up to larger columns, MabSelect PrismA resin is also available in bulk as well as prepacked in ReadyToProcess™ columns.

Antibody purification and homogeneity analysis

In monoclonal antibody (mAb) production and characterization, a critical step is the analysis of the final mAb product, and quantification of mAb aggregates is an important part. As the presence of aggregates in pharmaceutical products can cause immunogenicity, a low aggregate level is important. Figure 5A shows separation of a mAb on a 1 mL HiTrap MabSelect PrismA column, and results from analysis of monomers/aggregate content in the collected fractions by size exclusion chromatography (SEC) on a Superdex™ 200 Increase 10/300 GL column is displayed in Figure 5B.

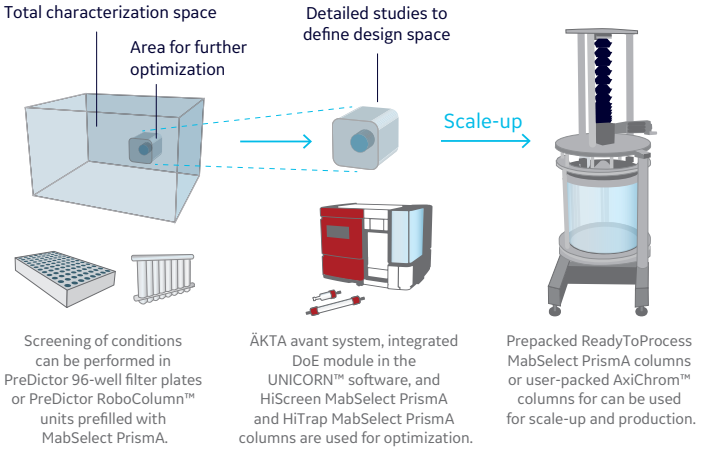
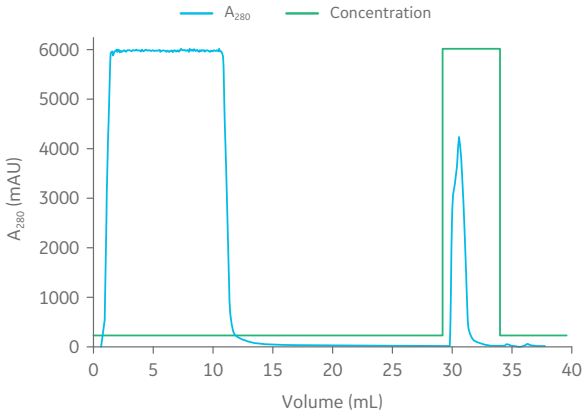


Fig 4. Process development workflow.

(A)

Column: 1 mL HiTrap MabSelect PrismA column
Sample load: 10 mL mAb (2.5 mg/mL)
Binding buffer: 20 mM phosphate, pH 7.4 + 150 mM NaCl
Elution buffer: 50 mM sodium acetate, pH 3.5
System: ÄKTA pure 25 chromatography system



(B)

Column: Superdex 200 Increase 10/300 GL column
Sample load: 100 µL mAb purified on MabSelect PrismA
Running buffer: 20 mM phosphate, pH 7.4 + 150 mM NaCl
System: ÄKTA pure 25 chromatography system

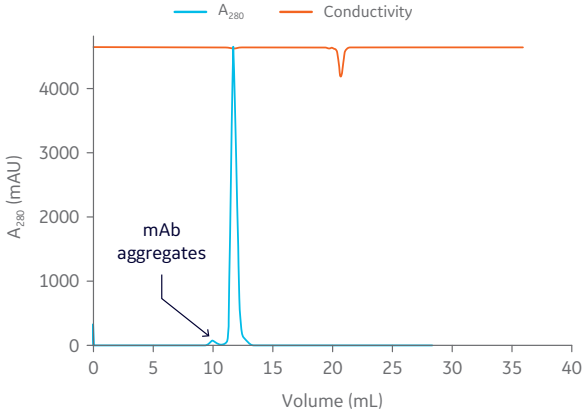


Fig 5. (A) Chromatogram from an analytical separation of mAb monomers from aggregates on a 1 mL HiTrap MabSelect PrismA column. (B) SEC analysis of collected fractions on a Superdex 200 Increase 10/300 GL column. By combining the antibody affinity step with high-resolution SEC, an understanding of the antibody profile can be obtained, which is important in the purification process.

Specifications

The main characteristics of the resin and columns are summarized in Tables 2 and 3, respectively. Note that HiTrap and HiScreen columns cannot be opened or repacked.

Table 2. Resin characteristics

	MabSelect PrismA
Matrix	Rigid, highly cross-linked agarose
Particle size, d_{50V}*	~ 60 µm
Ligand	MabSelect PrismA ligand (alkali-tolerant, protein A-derived from *E. coli*)
Coupling chemistry	Epoxy
Dynamic binding capacity, Q_{B10}†	~ 40 mg polyclonal IgG/mL resin, 2 min residence time‡ ~ 80 mg polyclonal IgG/mL resin, 6 min residence time§
Chemical stability	Stable in commonly used aqueous buffers for protein A chromatography
pH stability, operational¶	3 to 12
pH stability, CIP**	2 to 14
Temperature stability	2°C to 40°C
Storage	20% ethanol, 2°C to 8°C

* Median particle size of the cumulative volume distribution.

† Determined at 10% breakthrough by frontal analysis in a laboratory scale column in PBS buffer, pH 7.4.

‡ Flow rate 0.5 mL/min (78 cm/h) in a 1 mL HiTrap column with a 2.5 cm bed height.

§ Flow rate 0.8 mL/min (100 cm/h) in a HiScreen column with a 10 cm bed height.

¶ pH range where resin can be operated without significant change in function.

** pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

Table 3. Column characteristics

	HiScreen	**HiTrap, 1mL**	**HiTrap, 5 mL**
Column volume	4.7 mL	1 mL	5 mL
Column dimensions	0.77 × 10 cm	0.7 × 2.5 cm	1.6 × 2.5 cm
Column hardware pressure limit	8 bar (0.8 MPa)	5 bar (0.5 MPa)	5 bar (0.5 MPa)
Recommended operating flow rate*	1.8 mL/min	0.5 mL/min	2.5 mL/min
Maximum operating flow rate*	< 4.7 mL/min	< 4 mL/min	< 20 mL/min

* At room temperature in buffers with the same viscosity as water at 20°C.

Ordering information

Product	Size	Product code
HiTrap MabSelect PrismA	1 × 1 mL	17549851
	5 × 1 mL	17549852
	1 × 5 mL	17549853
	5 × 5 mL	17549854
HiScreen MabSelect PrismA	4.7 mL	17549815
Related products		
MabSelect PrismA resin	25 mL	17549801
MabSelect PrismA resin	200 mL	17549802
PreDictor MabSelect PrismA	6 µL	17549830
	20 µL	17549831
	50 µL	17549832
PreDictor RoboColumn MabSelect PrismA	200 µL	17549833
	600 µL	17549834
ReadyToProcess MabSelect PrismA	1 L	17549861
	2.5 L	17549862
Other products		
HiTrap Desalting	1 × 5 mL	29048684
	5 × 5 mL	17140801
Superdex 200 Increase 10/300 GL	24 mL	28990944

Accessories	Size	Product code
Union 1/16 inch male / 1/16 inch male (0.5 mm i.d.)	2	18112093
1/16 inch male / luer female*	2	18111251
Tubing connector flangeless / M6 female	2	18100368
Tubing connector flangeless / M6 male	2	18101798
Union 1/16 inch female / M6 male	6	18111257
Union M6 female / 1/16 inch male	5	18385801
Union luerlock female / M6 female	2	18102712
Male connector for ÄKTA systems, 1/16 inch	8	28401081
Stop plug female, 1/16 inch†	5	11000464
Fingertight stop plug, 1/16 inch‡	5	11000355

Related literature	
Data file: MabSelect PrismA	KA553200917DF
Data file: HiScreen prepacked columns	28930581

* One connector included in each HiTrap package.

† Two, five, or seven female stop plugs included in HiTrap packages depending on product.

‡ One fingertight stop plug is connected to the inlet and to the outlet of each HiScreen column, and to the top of each HiTrap column, at delivery.

gelifesciences.com/solutions/protein-research

GE Healthcare Bio-Sciences AB, Björkgatan 30, 751 84 Uppsala, Sweden
GE Healthcare Bio-Sciences Corp., 100 Results Way, Marlborough, MA 01752, USA
GE Healthcare Europe GmbH, Munzinger Strasse 5, D-79111 Freiburg, Germany
GE Healthcare Japan Corp., Sanken Bldg., 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan
GE Healthcare UK Ltd., Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK
For local office contact information, visit gelifesciences.com/contact.

KA3579250418DF