HiTrap Fibro PrismA units
HiScreen Fibro PrismA units

AFFINITY CHROMATOGRAPHY

More targeted patient populations and smaller manufacturing batch sizes are driving the demand for increased process efficiency and flexible multiproduct facilities in monoclonal antibody (mAb) manufacturing. To help meet these needs, we developed ready-to-use Fibro PrismA units (Fig 1) for capturing mAbs and F_c-containing recombinant proteins. Fibro PrismA units have a protein A cellulose fiber matrix with an open pore structure where mass transfer is governed by convective flow. This structure allows high mAb binding capacities at very short residence times, which results in cycle times of minutes instead of the hours needed for resin-based chromatography. The Fibro PrismA units can be used for up to 200 cycles before disposal, depending on the application.

With HiTrap™ and HiScreen™ Fibro PrismA units connected to ÄKTA™ system you can:

- Purify proteins quickly using rapid cycling chromatography. Cycle times are less than five minutes compared with hours for chromatography resins.
- Achieve high-throughput purification of up to 500 mAbs/wk for clone selection and lead candidate optimization. Each run/purification cycle has real-time UV, pH, and conductivity detection that generates a chromatogram.
- Increase throughput up to 20-fold over that for resin-based chromatography. This cuts weeks from process development lead times.
- Perform a full lifetime study in less than 24 hours.

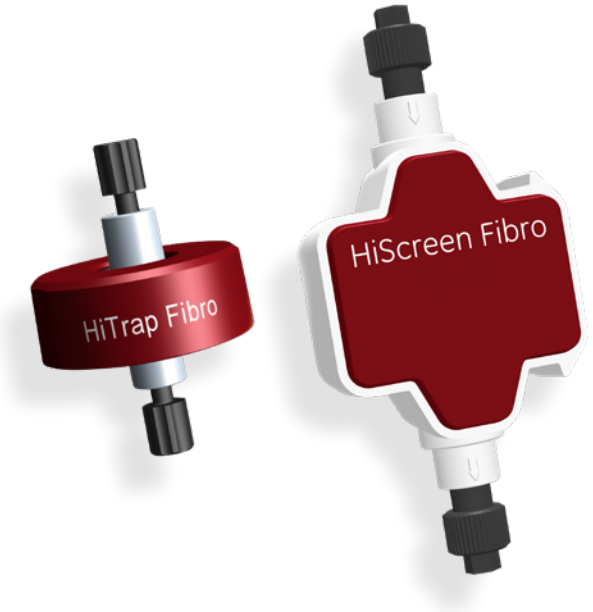


Fig 1. HiTrap and HiScreen Fibro PrismA units are delivered ready for use.

Product overview

Fibro chromatography, based on electrospun cellulose, offers a large surface area for high binding capacity. The matrix has an open structure with high mechanical strength, which allows high flow rates. Residence times are measured in seconds rather than the minutes required for resin-based chromatography.

The proprietary structure of the cellulose fibers allows the technology to overcome the diffusional and flow limitations of packed bed chromatography purification, as well as the capacity issues of membrane adsorbers and monoliths (Fig 2 and Fig 3). Fibro PrismA has the same engineered PrismA protein A ligand as the MabSelect™ PrismA chromatography resin. This assures optimized binding capacity and excellent alkaline resistance for efficient cleaning-in-place (CIP).

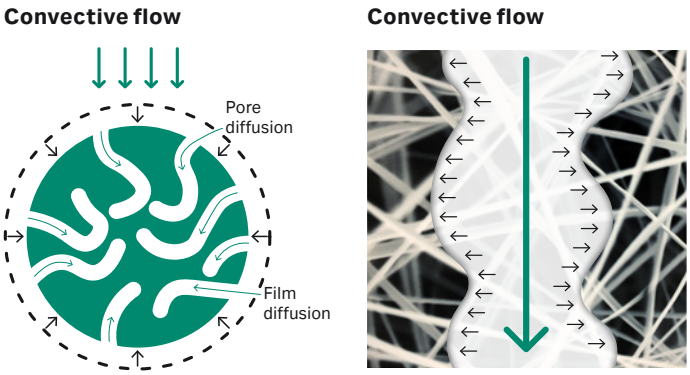
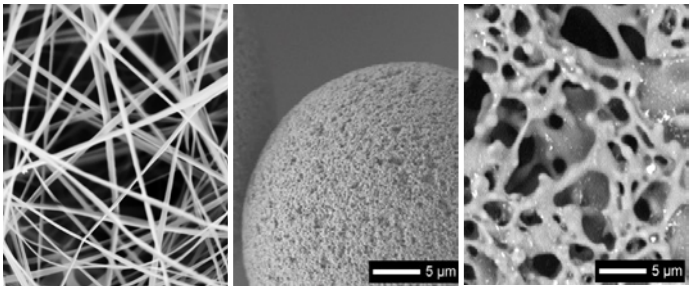


Fig 2. Flow rates in resin-based chromatography (left) are limited by diffusion. The open structure in Fibro fibers (right) allows convective flow and direct mass transfer of the target protein to the ligand immobilized on the fiber surface.



Fibro matrix
Convective mass transfer
Surface area ~ 10 m^2/g

Chromatography resins
Binding via diffusion into the particle
Surface area ~ 40 m^2/g

Membrane adsorbers
Convective mass transfer
Surface area ~ 0.9 m^2/g

Fig 3. Surface area and mass transfer mechanisms for different chromatography base matrices.

HiTrap and HiScreen Fibro PrismA units

These ready-to-use units are designed for research and early process development and are suitable for screening and optimization of process conditions. They are made of medical-grade polypropylene plastic with stoppers at the inlet and outlet.

You can operate both units with either a peristaltic pump or an ÄKTA chromatography system. The HiTrap Fibro unit can also be operated with a syringe and using bidirectional flow. For the smallest possible elution pool volume with the HiScreen Fibro unit, use the defined inlet and outlet to elute proteins in the defined flow direction.

The HiTrap Fibro PrismA unit has one bed, which allows for operation at very high flow rates of 40 matrix volumes (MV)/min on an ÄKTA system. The HiScreen Fibro PrismA unit has two parallel beds that are each twice the thickness of the bed in the HiTrap format (Fig 4). Thus, the recommended flow rate for HiScreen Fibro is lower (8 MV/min) than the flow rate for the HiTrap format.

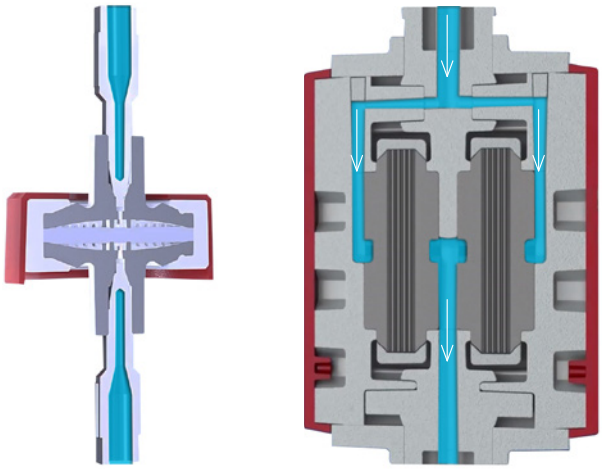


Fig 4. Cross-sectional image of HiTrap (left) and HiScreen (right) Fibro PrismA units. The bed design in the forthcoming large-scale Fibro PrismA units will be similar to HiScreen Fibro PrismA, but the flow path design will differ.

The characteristics of HiTrap and HiScreen Fibro PrismA units are summarized in Table 1.

Table 1. Main characteristics for HiTrap and HiScreen Fibro PrismA units

	HiTrap Fibro PrismA	HiScreen Fibro PrismA
Matrix	Derivatized electrospun cellulose fibers	
Ligand	PrismA ligand (alkali-stabilized protein A derived from *E. coli*)	
Ligand coupling	Single point attachment	
Dynamic binding capacity (DBC)[1]	~ 30 mg IgG/mL matrix	
Typical DBC/unit[1]	~ 12 mg IgG/HiTrap unit	~ 112 mg IgG/HiScreen unit
Cycle time[2]	~ 3 min	~ 5 min
Flow, recommended operating[3]	≤ 16 mL/min (40 MV/min)	≤ 30 mL/min (8 MV/min)
Matrix volume	0.4 mL	3.75 mL
Elution volumes[4]	≤ 7 MV	≤ 4 MV
Maximum operating pressure	1 MPa (10 bar)	
Chemical stability	Compatible with aqueous buffers commonly used for protein A chromatography	
pH stability, operational[5]	3 to 12	
pH stability, CIP[6]	2 to 14	
Temperature stability, operational	4°C to 35°C	
Temperature stability, storage	2°C to 8°C	
Storage	20% v/v ethanol in water	

[1] Determined at 10% breakthrough by frontal analysis in Tris buffer, pH 7.5.
[2] Purification cycle including steps of: equilibration, sample loading, washing, elution, strip, CIP and reequilibration of the Fibro unit.
[3] At room temperature using a buffer with the same viscosity as water.
[4] HiTrap Fibro and HiScreen Fibro unit housing designs have not been optimized for small elution volumes. But the forthcoming process-scale good manufacturing practices (GMP) compatible Fibro units will be. Elution volumes are expected to be < 3 MV.
[5] pH range where resin can be operated without significant change in function.
[6] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

The PrismA ligand

The electrospun cellulose fibers in Fibro are coupled with PrismA protein A ligand, which is produced in *E. coli*. Fermentation and subsequent purification are performed in the absence of animal products. The ligand has been specifically engineered for enhanced alkali and protease stability. The specificity of binding to the F_c region of IgG is similar to that of the conventional protein A ligand and provides excellent purification in one step. The PrismA ligand also has affinity for the VH3 chain, so it can be used to purify certain types of antibody fragments.

High binding capacity at very short residence times

The macroporosity and large surface area of the Fibro matrix allow very fast purification with residence times in seconds, not the minutes required with resin-based chromatography (Fig 5). This means that mAbs can be purified up to 20 times faster than with resin-based chromatography. A full mAb purification cycle with equilibration, loading, washes, elution, CIP, and re-equilibration can be performed in minutes rather than hours, as shown in Figure 6.

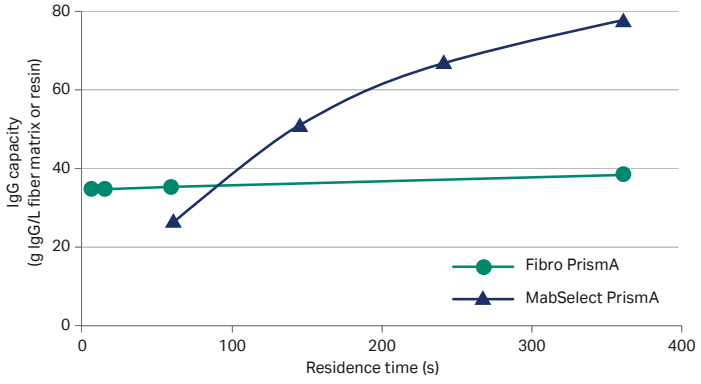


Fig 5. The immediate mass transfer of the Fibro matrix enables high binding capacity at very short residence times.

The large surface area and binding properties of the PrismA ligand enable high dynamic binding capacities of approximately 30 g/L, see Figure 7. Traditional mAbs as well as antibody variants have been evaluated and have high capacities. As shown in Figure 7, the dynamic binding capacity varies for different molecules.

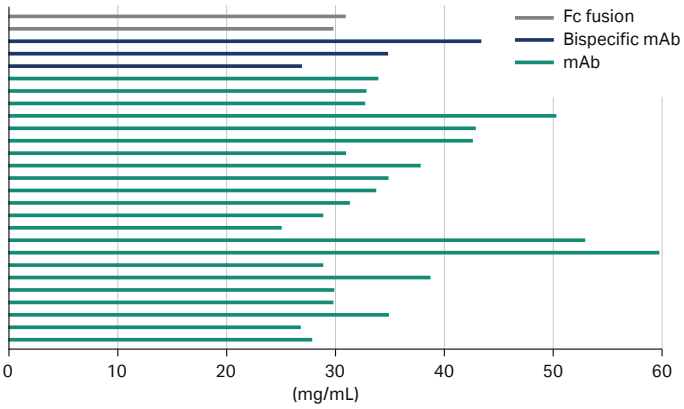


Fig 7. Dynamic binding capacity at < 6 s residence time of Fibro PrismA matrix for a variety of mAbs and antibody variants.

High alkaline stability

In research applications when different types of antibodies are purified on the same unit, it is important to prevent cross-contamination while maintaining recovery. Cleaning is also important when re-using a unit over a lifetime of hundreds of cycles. Sodium hydroxide (NaOH) is an efficient, low-cost, and easy-to-dispose reagent when thorough cleaning is required. Rigorous cleaning with NaOH 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.

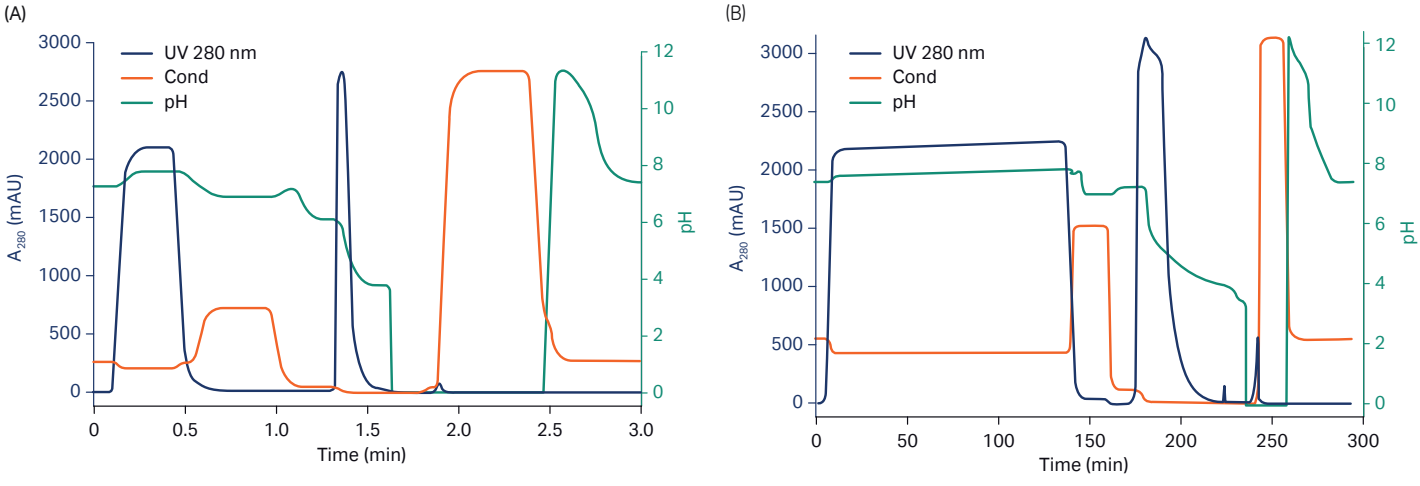


Fig 6. Typical purification cycle for high capacity loads using (A) a HiTrap Fibro PrismA unit and (B) a HiTrap 1 mL column containing MabSelect PrismA resin.

With the enhanced alkaline stability of the PrismA protein A ligand, the recommended CIP is 0.5 to 1.0 M NaOH for 30 s to 1 min in every cycle. This means that HiTrap and HiScreen Fibro PrismA units can be confidently cleaned for reuse. If needed Fibro PrismA units can be cleaned with up to 2 M NaOH over hundreds of cycles while still retaining a high binding capacity (Fig 8).

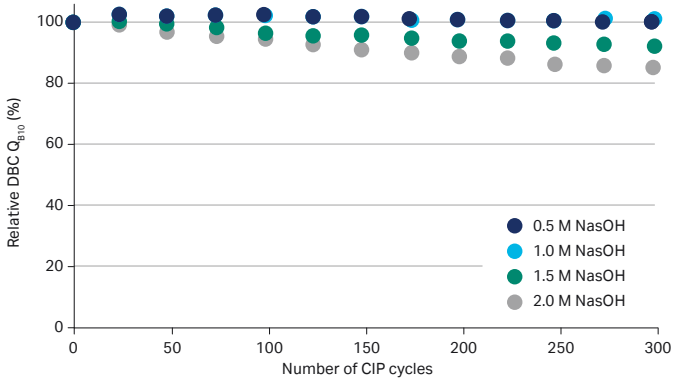


Fig 8. Relative remaining dynamic binding capacity of HiTrap Fibro PrismA after CIP for 300 cycles using 0.5 to 2.0 M NaOH for 1 min between runs.

Similar purification performance to resin-based columns

A CHO (Chinese hamster ovary) cell culture supernatant sample containing mAb 1 was purified using a HiTrap Fibro PrismA unit (0.4 mL) and a HiTrap MabSelect PrismA column (1 mL) on an ÄKTA pure 25 system. Performance of the Fibro PrismA unit was comparable to that of the column, as shown by similar recovery (Fig 9), host cell protein (HCP) removal (Fig 10), aggregate concentration (Fig 11), and protein A leakage (Fig 12).

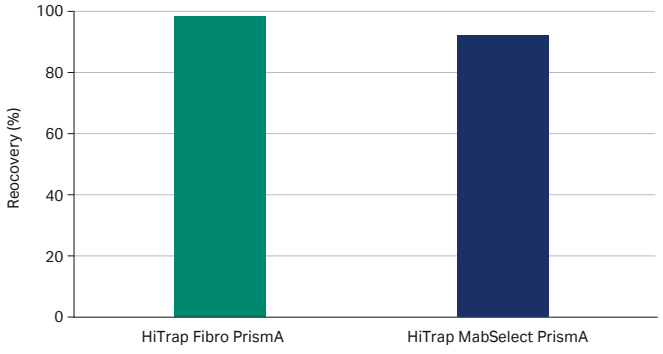


Fig 9. Recovery of mAb 1 after purification on HiTrap Fibro PrismA or HiTrap MabSelect PrismA.

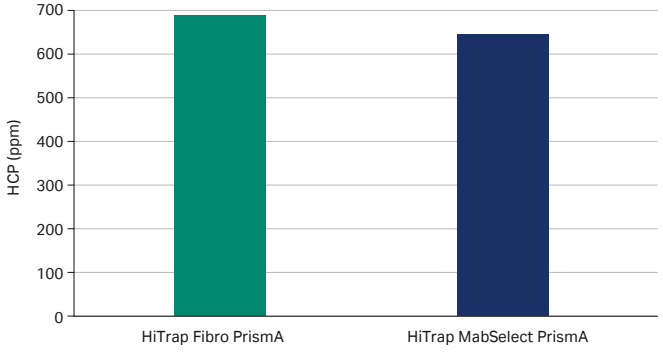


Fig 10. Remaining HCP (ppm) in the elution pool after purification of mAb 1. HCP in loaded feed was approximately 170 000 ppm.

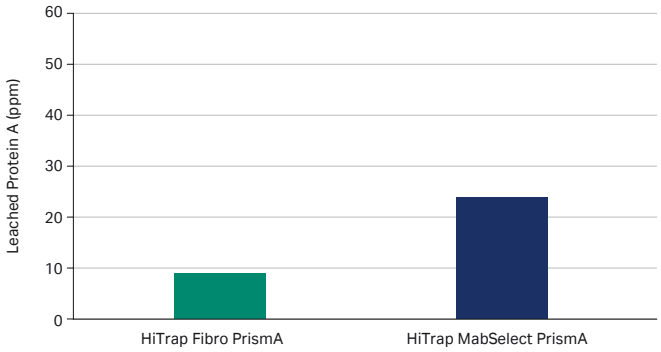


Fig 11. Leached protein A (ppm) after purification of mAb 1. The slightly increased level observed for MabSelect PrismA is a result of the higher ligand density compared with the Fibro PrismA unit.

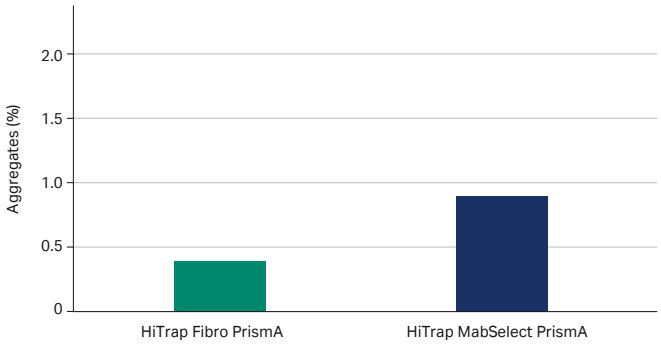


Fig 12. Aggregate levels (%) after purification of mAb 1.

General recommendations for cell culture pre-treatment when using Fibro PrismA

Fibro PrismA cycle lifetime is directly related to the properties and volume of the mAb harvest being loaded onto the Fibro PrismA unit. There are multiple examples where > 200 cycles have been successfully performed with well clarified feeds. In general, mAb harvests should be clarified by centrifugation followed by a polishing depth filter, and finally sterile filtration (0.2 µm). Clarification can also be done using a filter train including a coarse depth filter for cell removal, followed by a fine depth filter for polishing, and finally sterile filtration (Table 2). Depth filtration efficiently removes process related impurities that can lead to fouling of the Fibro PrismA matrix.

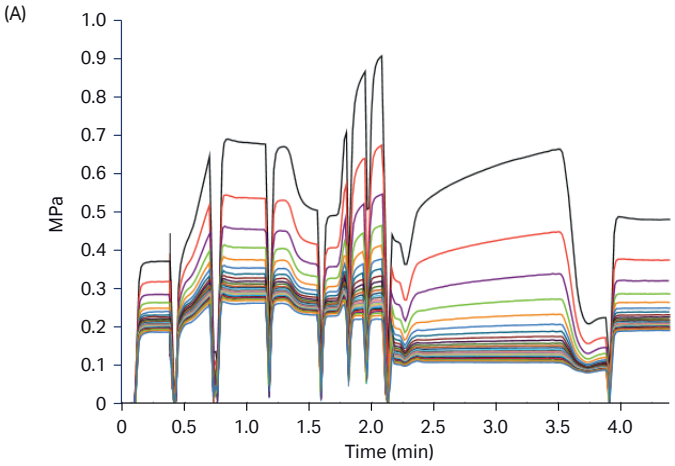
Clarified harvests should be processed as soon as possible after clarification. If that is not possible, the harvest should be frozen after clarification, thawed, and 0.2 µm filtered immediately before processing.

For sample preparation in research applications where small volumes are used, diatomaceous earth (DE) is recommended to be added to the sample followed by 0.2 µm filtration using a bottle-top filter.

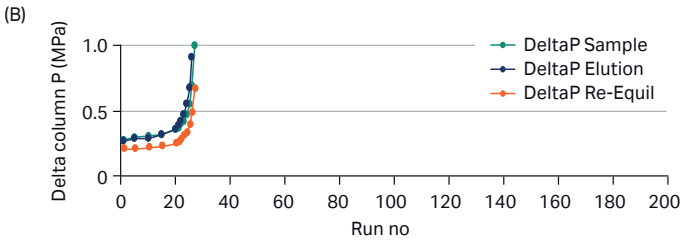
Table 2. Suggested clarification process of cell culture material prior to using Fibro PrismA

Step	Centrifugation/filtration	Filter train
Cell removal	Centrifugation	Coarse depth filter
Polishing	Fine depth filter	Fine depth filter
Sterile filtration	0.22 µm filter	

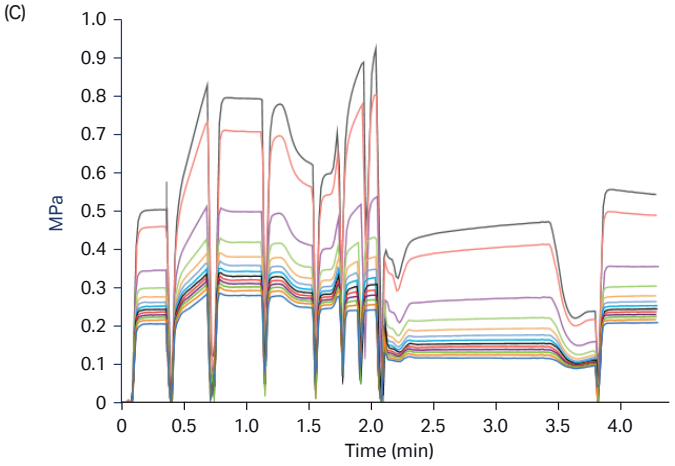
In cases of challenging materials, a charged depth filter can be used in addition to the general harvest procedure or as a replacement of the fine depth filter step. This will give an more efficient removal of charged particles like DNA/HCP and/or chromatin. See below an example of a challenging mAb harvest where depth filtration prolonged the lifetime of the Fibro PrismA unit significantly. Including a charged depth filter step increased lifetime of the Fibro PrismA unit and only a small pressure increase over the 200 cycles was observed.



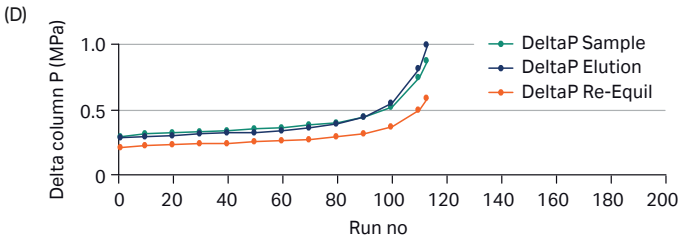
Overlay of delta pressure increase of untreated mAb feed over all 27 cycles



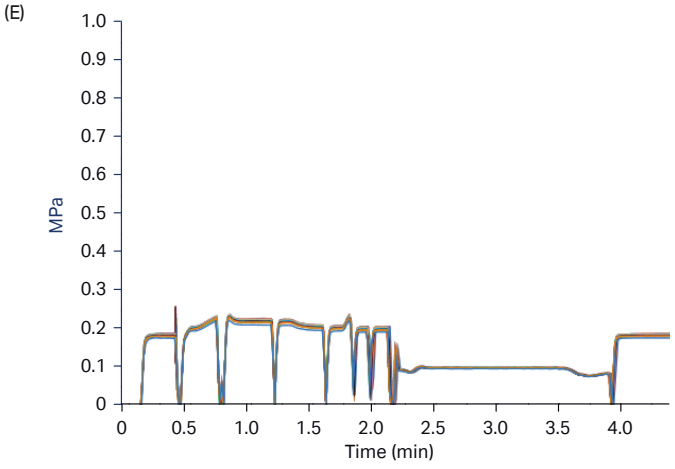
Delta pressure increase of untreated mAb feed over cycles



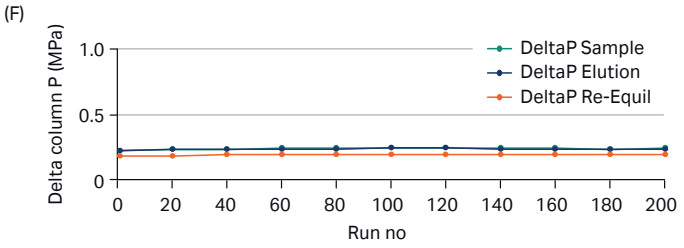
Overlay of delta pressure every tenth cycle, and the last cycle for mAb feed pretreated with PDE2 depth filter over 113 cycles



Delta pressure increase of mAb feed pretreated with PDE2 depth filter



Overlay of the delta pressure increase of every 10 cycles , over 113 cycles, for mAb feed pretreated with charged depth filter.



Delta pressure of mAb feed pretreated with charged depth filter.

Fig 13. HiTrap Fibro PrismA lifetime increased with the use of depth filtration for a challenging mAb harvest.

HiScreen Fibro PrismA rapid cycling performance

HiScreen Fibro PrismA is optimal for early process development and lifetime studies. As an example, a mAb harvest with a titer of 2.1 g/L was pretreated with charged depth filter. It was then purified on a HiScreen Fibro PrismA unit over 200 cycles using an ÄKTA pure 150 system. The load was approximately 24 mg/mL, corresponding to 80% of the QB10% value of 30 mg/mL. Cleaning-in-place (CIP) was done with 0.5 M NaOH at a contact time of 1 min/cycle. The flow rate was 30 mL/min, i.e., 8 MV/min except for the CIP which was done at half the flow rate. The eluate was collected after every 50 cycles and analyzed for mAb and Host Cell Protein (HCP) concentration, as well as, PrismA protein A ligand leakage in order to visualize the purification performance over the cycle lifetime. The UV profile was consistent over the cycles (Fig 14 A) and a very small pressure increase was observed (Fig 14 B).

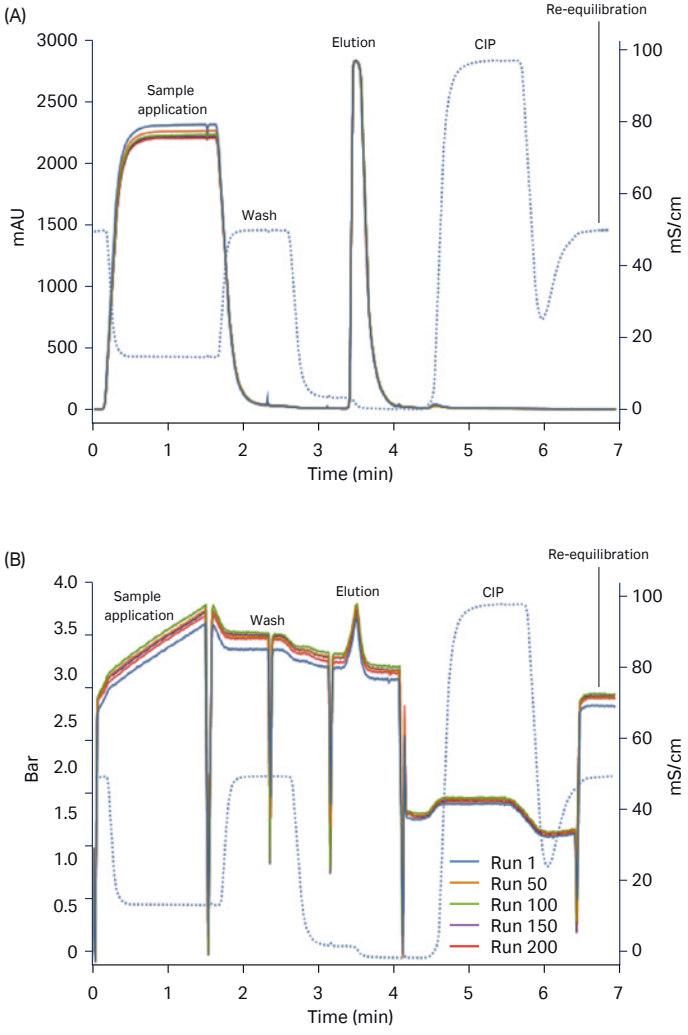


Fig 14. (A) Overlay of UV280 nm every 50 cycles (1, 50, 100, 150, and 200) from HiScreen Fibro PrismA lifetime study. (B) Overlay of dCP every 50 cycles (1, 50, 100, 150 and 200) from HiScreen Fibro PrismA lifetime study.

The same mAb harvest material was also purified on a HiTrap Fibro PrismA unit operated on an ÄKTA pure 25 system using the same buffer conditions as for the HiScreen Fibro PrismA unit. The flow rate for the HiTrap Fibro PrismA unit was 16 mL/min (40 MV/min). Also for HiTrap Fibro PrismA, the mAb load was 80% of QB10% and fractions were collected every 50 cycles for trending of purification performance.

The yield for both Fibro PrismA formats was close to 90% (Fig 15). The HCP concentration was in the range of 200 to 300 ppm and was consistent between the Fibro PrismA formats (Fig 16). The PrismA ligand leakage was approximately 13 ppm in the first cycle but < 5 ppm in the other eluates (Fig 17). The elution volume was in the range of 4.4 to 4.8 MV for HiTrap Fibro PrismA and 3.3 to 3.5 MV for HiScreen Fibro PrismA (Fig 18). The elution volumes from the small-scale Fibro PrismA units are larger than the elution volumes from the future pilot and process scale Fibro PrismA units where the flow path has been further optimized.

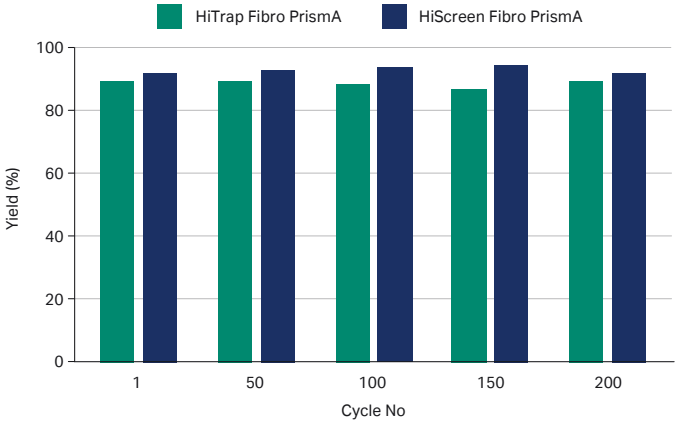


Fig 15. HiTrap Fibro PrismA and HiScreen Fibro PrismA yield over lifetime study.

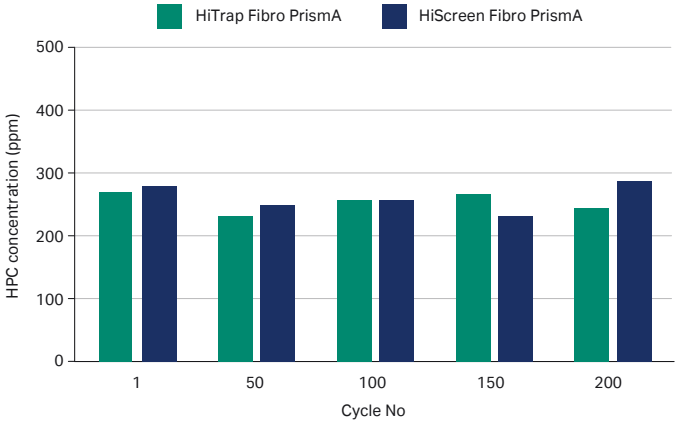


Fig 16. HiTrap Fibro PrismA and HiScreen Fibro PrismA HCP clearance over lifetime study.

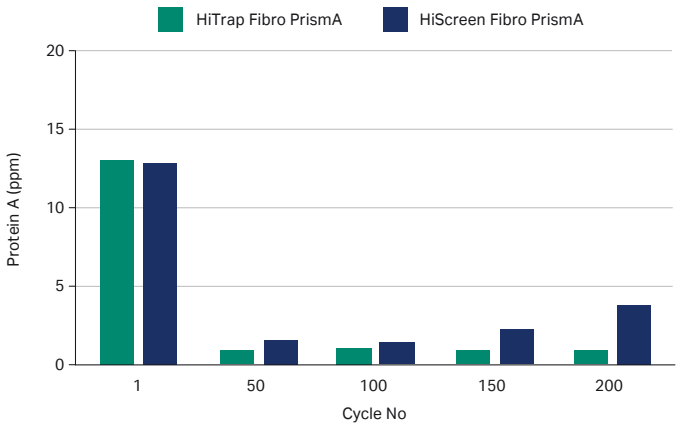


Fig 17. HiTrap Fibro PrismA and HiScreen Fibro PrismA ligand leakage over lifetime study.

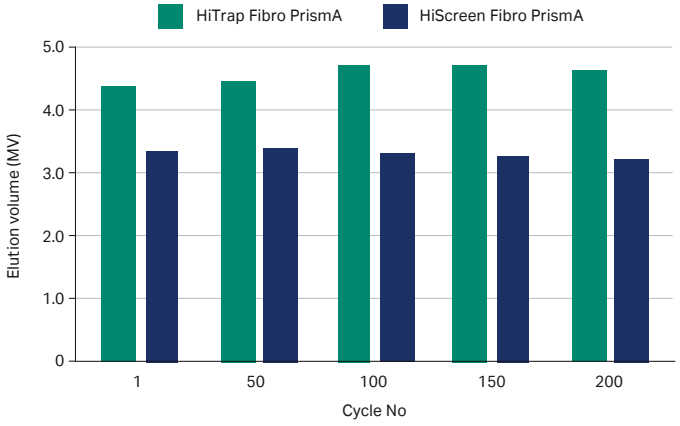


Fig 18. HiTrap Fibro PrismA and HiScreen Fibro PrismA elution volume over lifetime study.

Fibro PrismA: a scalable protein A fiber chromatography platform

The Fibro technology platform is designed for scalability up to manufacturing scale and compatibility with existing ÄKTA purification systems. The units will range from research scale with a capacity of approximately 12 mg mAb per purification cycle (HiTrap units) to good manufacturing practices (GMP) compatible units that can process up to 2000 L bioreactor harvest, or more than 10 kg mAb in less than 24 hours by cycling multiple times.

Short cycle times enable high-throughput purification of mAbs and opportunities for substantial time savings during research and process development. At larger scales in the biomanufacturing process, operation in a rapid cycling manner allows full utilization of the protein A lifetime during one batch, as well as cost-effective single-use chromatography.

In an independent evaluation, a process-scale Fibro PrismA prototype unit (0.6 L) was run for 17 cycles on an ÄKTA ready chromatography system equipped with a High Flow kit. The results showed good performance with DBC of 30.6 g/L and cycle times of 7.3 min with 2.8 bar max delta column pressure (dCP) and 4.8 min with 4.2 bar max dCP. Eluate volumes were below 3 MV at > 95% recovery. Buffer usage was 0.65 L/g (~ 18 MV per run). As shown in Figure 19, performance was maintained across cycles.

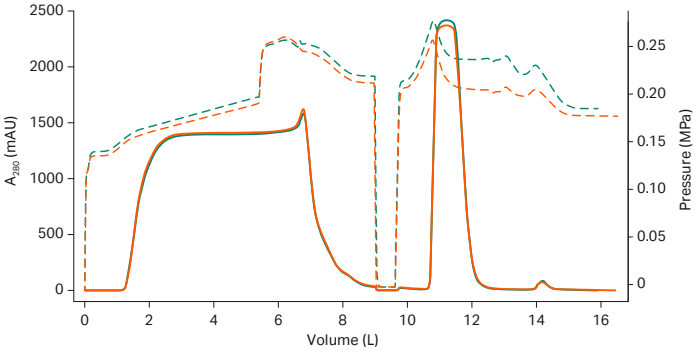


Fig 19. Overlaid chromatograms of cycles 1 and 13 of Fibro PrismA large-scale (0.6 L) prototype unit.

Fibro units are compatible with existing ÄKTA chromatography systems. We recommend using HiTrap Fibro PrismA with ÄKTA pure/avant 25 and 150. For HiScreen Fibro PrismA we recommend ÄKTA pure/avant 150. Instrument configurations with predefined UNICORN™ chromatography methods for Fibro are available for these systems. HiTrap and HiScreen Fibro units can also be run on other ÄKTA systems, as shown in Table 2. The forthcoming large-scale GMP compatible Fibro PrismA units will be compatible with ÄKTA pilot 600 system, ÄKTAprocess™ system, and ÄKTA ready system.

Storage

Store HiTrap and HiScreen Fibro PrismA in 20% ethanol at 2°C to 8°C.

Before use, equilibrate with binding buffer and perform a blank run, including CIP.

Table 3. Fibro units and ÄKTA system compatibility based on recommended flow for each Fibro unit on each system. Blue = preferred system, gray = compatible but not optimal

System	Chromatography system		HiTrap Fibro PrismA (0.4 mL)	HiScreen Fibro PrismA (3.75 mL)
	Min flow (mL/min)	Max flow (mL/min)	Recommended flow, mL/min	Recommended flow, mL/min
ÄKTA go	0.01	25	16	25
ÄKTA pure/avant 25	0.001	25	16	25
ÄKTA pure/avant 150	0.01	150	16	30

Ordering information

Product	Column size	Product code
HiTrap Fibro PrismA 1 pack	0.4 mL	17549855
HiTrap Fibro PrismA 4 pack	4 × 0.4 mL	17549856
HiScreen Fibro PrismA	3.75 mL	17549816

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