# High-throughput and single-use mAb purification with Fibro chromatography

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## Introduction

Biomanufacturing is trending towards increased number of monoclonal antibody (mAb) projects and smaller batch sizes, with production of most mAbs expected to be below 150 kg/yr*. This puts new demands on number of clones to be screened, efficient process development (PD), and flexible, scalable bioprocess manufacturing design. In multiproduct facilities easy and fast changeover between campaigns is becoming essential. This is driving adoption of efficient single-use technologies.

Here, we present a technology that brings new capabilities to address these needs. Rapid cycling, fiber-based chromatography (Fibro) enables substantially reduced purification times in research and PD and a single-use solution in manufacturing. Fibro chromatography supports relatively high capacity with residence times of seconds and allows full chromatographic runs in a few minutes per cycle. We describe a versatile solution that substantially increases throughput in research and PD and maintains consistency over cycles. A large-scale prototype run demonstrates consistent performance over cycles and scalability.

## What is Fibro chromatography?

The Fibro adsorbent material has a cellulose fiber matrix with an open pore structure where mass transfer is governed by convective flow. This allows for high binding capacity (> 30 g/L) and residence times measured in seconds rather than minutes (Fig 1 and 2).

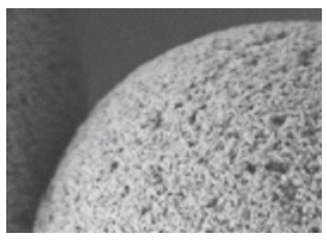

**Fig 1.** Conventional chromatography: diffusive flow. Mass transfer is restricted by the slow diffusion of molecules through the pores in the beads. Process flow is restricted by bead rigidity.

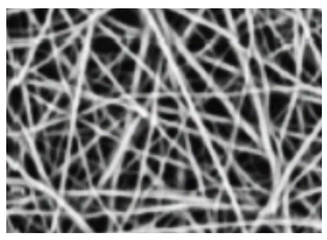

**Fig 2.** Fibro chromatography: convective flow. Fast mass transfer by convective flow. Process flow is restricted only by the size of the pores in the matrix material.

## Large-scale Fibro PrismA prototype run – shows concentrated elution, consistency over cycles, scalability

A 600 mL large-scale Fibro PrismA prototype unit was evaluated by an independent collaborator. Using an ÄKTA ready system fitted with an ÄKTA ready High Flow Kit, 17 cycles were run with clarified mAb feed harvest (Fig 7). The dynamic binding capacity was measured at 30.6 g/L, and the cycling time was 7.3 min with 2.8 bar max CdP and 4.8 min with 4.2 bar max CdP. The recovery was > 95%, and the elution pool volume obtained was below 2.45 MV measured by 100 mAU/100 mAU UV absorbance cut off collection. The buffer consumption was 0.65 L/g (~ 18 MV per run), which is comparable to a protein A resin column.

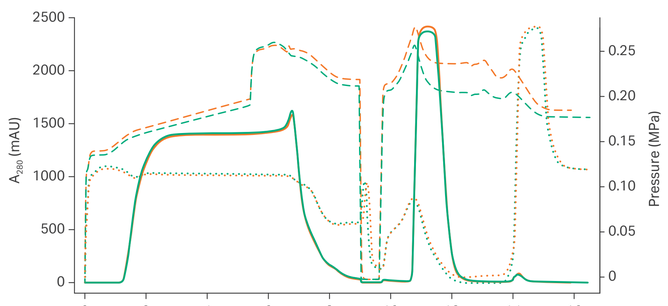


**Fig 7.** Overlaid chromatograms: cycles 1 and 13 of Fibro PrismA prototype 600 mL unit.

## High binding capacity at short residence time and consistency over cycles

A scalable 4 mL Fibro unit immobilized with the protein A "PrismA" ligand can run a full chromatographic cycle in a few minutes with concentrated elution pool volumes of less than 3 matrix volumes (MV) (Fig 3). Consistent performance over cycles (recovery > 95%) was demonstrated by running 50 cycles with the 4 mL Fibro PrismA unit on ÄKTA™ pure 150 system (Fig 4). The elution peak has the same behavior over cycles and the pressure is stable.

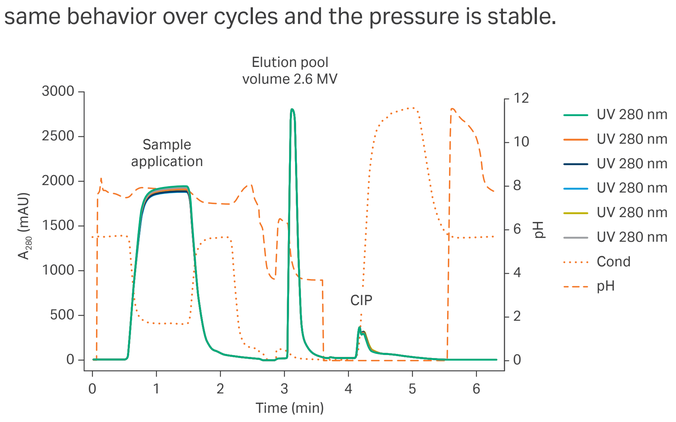


**Fig 3.** Protein A bind-elute profile on clarified cell culture mAb feed of 3.8 mg/mL purified on a ~ 4 mL Fibro PrismA unit: Overlay $UV_{280}$ nm every 10th cycle, cycle time 6.3 min, flow rate 8 MV (CIP 4 MV).

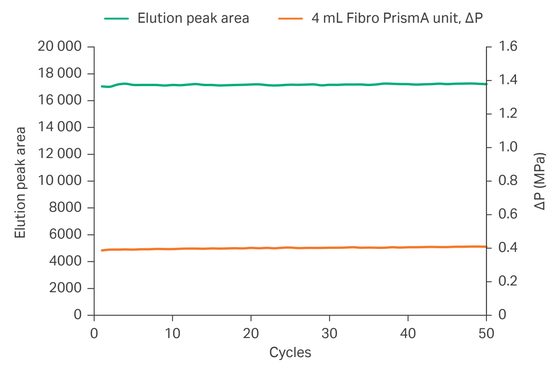


**Fig 4.** Trend curves of max delta column pressure (ΔP) and area of elution peak performance over cycles.

## High throughput, automated one- and two-step purification of mAb samples with laboratory-scale HiTrap™ Fibro PrismA unit

When screening multiple clones or optimizing a process, it is critical to have automated, efficient purification solutions with minimized cross-contamination risk.

Purification of 10 different mAb samples was set up on ÄKTA pure (Fig 5) with a Teledyne™ autosampler. The 0.4 mL HiTrap Fibro PrismA unit was compared with a 1 mL HiTrap MabSelect™ PrismA column. To check for carryover between runs of different samples, an SDS-PAGE analysis was performed on blank runs after the protein A step (Fig 6). No protein bands were visible, indicating lack of carryover. With the HiTrap Fibro PrismA unit, the capture step time was reduced from ~ 60 min to ~ 10 min (including ~ 5 min to clean and prepare the autosampler between samples), with similar recovery and purity to the HiTrap column.

An automated 2-step tandem purification of mAb feed samples was set up with the captured peak from the HiTrap Fibro PrismA unit directly transferred to 2 × 5 mL HiTrap Desalting columns. The setup included a versatile valve and a second UV to track sample from the second step. High recovery between the capture and the desalting steps was obtained, and cycle time was reduced more than 3-fold compared with HiTrap MabSelect PrismA (Table 1).

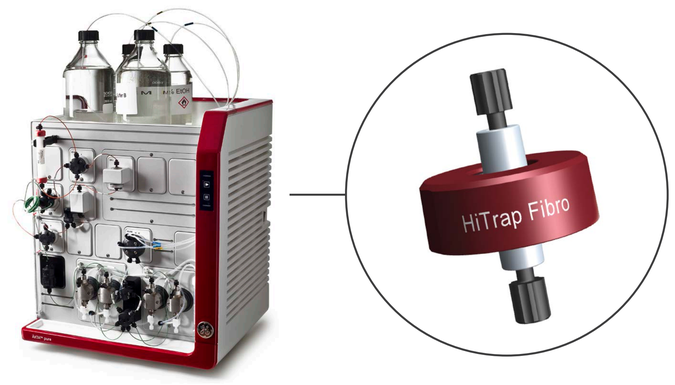


**Fig 5.** This picture illustrates ÄKTA pure and the HiTrap Fibro PrismA unit.

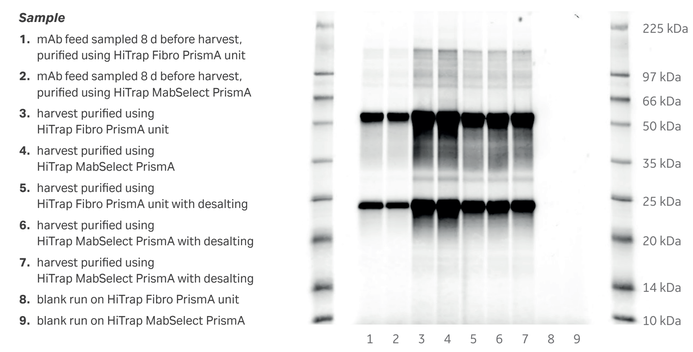


**Fig 6.** SDS-PAGE analysis after protein A step. Blank runs are samples 8 and 9.

**Table 1.** Recovery and run time of 2-step tandem purification

| Purification format | Recovery – UV measuring elution area of affinity step (mL × mAU) | Recovery – UV measuring elution area of desalting step (mL × mAU) | Total run time, including autosampler and CIP (min) |
|---|---|---|---|
| HiTrap MabSelect PrismA column, 1 mL | 1036 | 1027 | 84 |
| HiTrap Fibro PrismA unit, 0.4 mL | 1175 | 1109 | 22 |

## Conclusions

Here we describe a technology with the potential to reduce time-to-market and enable a cost-effective, single-use manufacturing solution:

- The open porous structure in the fiber adsorbent material enables purification cycle times of a few minutes.
- Serial set-up with autosampler on ÄKTA systems provides full chromatograms in high-throughput mode, which automates purification of large numbers of samples.
- Large scale Fibro PrismA prototype was successfully evaluated by an independent collaborator demonstrating concentrated elution pools, quick cycling time and low buffer consumption.

* Data derived from BDO's BioProcess Technology Consultants bioTRAK™ database.

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