Simplifying Progress

Does Size Matter? Purifying a Large Fc-Fusion Protein Using Sartobind® Rapid A

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1. Introduction

In the field of antibody purification, there is an increasing demand for high-performance chromatography membranes that are supplied ready-to-use and available in a scalable format, addressing purification bottlenecks from discovery to commercial manufacturing. The Sartobind® Rapid A technology increases productivity more than 10-fold compared to traditional resins (203 g/L/hr vs. 14 g/L/hr, respectively)[1]. This membrane also shows similar performance for dynamic binding capacity (DBC), yield, and host cell protein (HCP) | host cell DNA (hcDNA) removal. As such, the Sartobind® Rapid A supports a new generation of membrane-based purification platforms, driving efficiency in development and scale up workflows, accelerating time to clinic.

2. Membrane Chromatography

Resins bind small molecules effectively at low flow rates but are limited by diffusion. In contrast, membranes maintain consistent binding for various molecule sizes across broad flow rates and can handle flows up to 20 times higher than resins.[2] This makes membranes ideal for capturing large molecules like viruses and for polishing applications. To match these flow rates with packed bed columns (resins), column diameters must be increased, leading to unnecessarily large columns and excess capacity.[2]

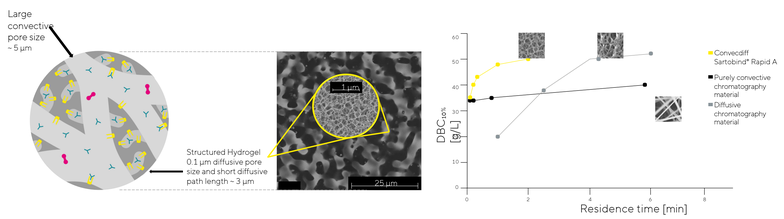


Figure 1: Sartobind® Rapid A Pore Structure, schematic (left) and electron microscopy picture (center). $DBC_{10\%}$ as a function of residence time for commercially available materials (purely diffusive or convective) and Sartobind® Rapid A (right)

Sartobind® Rapid A provides an alternative purification platform that improves overall process performance, especially in cases where the resin capacity remains underutilized. Thanks to the flow properties of the agarose-based membrane, it combines diffusive and convective mass transport.[3] It is able to purify antibodies while maintaining CQAs equivalent to those obtained with resin-based media and the equivalence of base-material opens the field, not only for substitutions in situations of delivery bottlenecks, but to consider it from the start as the most economical alternative.[3]

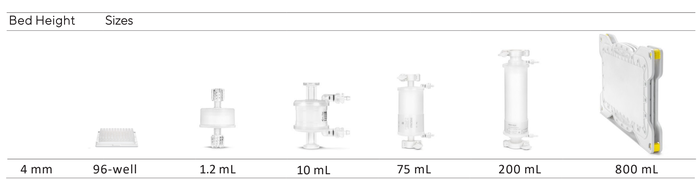


Figure 2: *Complete portfolio of Sartobind® Rapid A technology. 96-well and 1.2 mL devices enable high-throughput screening. Portfolio is linearly scalable from 1.2 mL to 800 mL devices, enabling seamless translation to larger formats and processing scales.*

3. Methodology and Experimental Design

A clarified harvest of a **545 kDa Fc-fusion protein** with a pI of 8.9 was used for all experimentation. The harvest was provided by an industry client seeking a more scalable and efficient protein purification process for their molecule. Past purifications using traditional Protein A resins resulted in low productivity and process efficiency due to the mass transfer limitations of large molecule diffusion into resin beads. Comparisons in performance, productivity, and efficiency of resin-based media and membrane adsorbers will be made.

Table 1: Comparison of Protein A resin and membranes used for side-by-side comparison

	Residence Time	Bed Volume	Observations
Sartobind® Rapid A (Sartorius)	30 sec	1.2 mL capsule	Chromatography membrane with protein A ligand
MabSelect PrismA™ (Cytiva)	6 min	HiTrap 1.0 mL column	Protein A resin - industry-standard, high-performing

Process steps for both the membrane- and resin-based purification technologies were developed and described in Table 1 based on vendor recommendations. Analytical methods were then developed and for testing same harvest material (CCH) and process step pools (Table 2).

Table 2: *Supporting analytics techniques and materials used to assess purification performance and efficiency*

	Column/Gel/Kit	Purpose	Comments
SDS-PAGE	3–8% Criterion XT Tris-Acetate	Relative purity	Optimal gel for large protein quantification
SE-HPLC	ShodexKW-803 Column, 5 µm, 8 mm x 300 mm	Aggregate Analysis	N/A
Protein-A HPLC	Sartorius CIMac r-Protein A 0.1ml	Titer Analysis	Optimal for large biomolecules

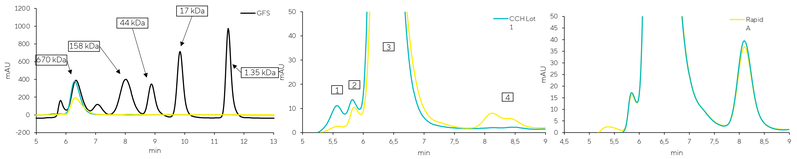
Preparative chromatography methods (Table 3) were developed using industry standard guidance aligned with vendor recommendations for the Protein A media in use.

Table 3: Preparative chromatography methods used for both Sartobind Rapid A and MabSelect PrismA experimentation

Step	Buffer	Sartobind® Rapid A Duration (CV)	RT	MabSelect PrismA™ Duration (CV)	RT
Equilibration	25 mM Tris, 100 mM NaCl, pH 7.4	10	6 sec	5	2 min
Loading	Clarified Harvest	Varies	30 sec	Varies	6 min
Post-load Wash	25 mM Tris, 100 mM NaCl, pH 7.4	1 10	30 sec 6 sec	1 5	6 min 2 min
Elution	100 mM Acetate, pH 3.0	10	12 sec	5	2 min
Neutralization	25 mM Tris, 100 mM NaCl, pH 7.4	10	6 sec	3	2 min
Regeneration	0.2 M NaOH (Rapid A) 0.1 M NaOH (Prisma)	10	12 sec	3 + 15 min static hold	2 min
Equilibration	25 mM Tris, 100 mM NaCl, pH 7.4	10	6 sec	5	2 min

4. Results

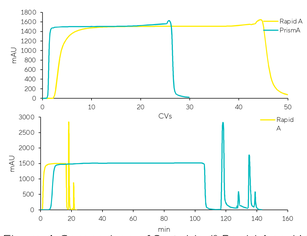
SDS-PAGE and SE-HPLC confirmed the molecular weight of the target molecule and also confirmed good impurity resolution when compared to a gel filtration standard (Bio-Rad). SE-HPLC appears to be comparable between Sartobind Rapid A and MabSelect PrismA.



	% Peak 1 5.4 min	% Peak 2 5.8 min	% Peak 3 6.3 min	% Peak 4 8.1 min
Purified Reference Lot #1	0.7 %	0.6%	98.6%	0.0%
Purified Reference Lot #2	0.8 %	2.1%	93.9%	3.1%

Figure 3: SEC data (top left and center) confirmed the molecular weight of the target molecule and good impurity resolution. SE-HPLC showed comparable purity for Sartobind Rapid A vs. MabSelect PrismA (top right), peak integration data not shown.

Higher capacity (193%) was observed with Sartobind® Rapid A vs. MabSelect PrismA™ using clarified harvest material at a titer of 1.16 g/L, attributed to the Rapid A's large convective pore structure. Yield, speed, and capacity were notably higher on the Sartobind® Rapid A membrane. Cycle time was reduced to 16% and yield increase by 28% when compared to MabSelect PrismA.

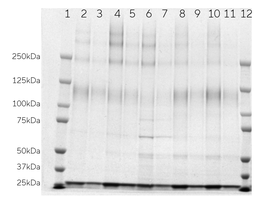


	Load RT (min)	$DBC_{10\%}$ (g/L)
Sartobind® Rapid A	0.5	43.2
MabSelect PrismA™	6	22.3

	Cycle Time (min)	Elution vol. (CV)	Yield Change
Sartobind® Rapid A	23	3.9	+ 28.3%
MabSelect PrismA™	159	1.6	-

Figure 4: Comparison of Sartobind® Rapid A and MabSelect PrismA™. $DBC_{10\%}$ (top) and purification cycle (bottom)

Comparable purity via SDS-PAGE is shown between Sartobind® Rapid A and MabSelect PrismA™. Further analysis will be completed to confirm purity and quality attributes are consistent between the two purification technologies chosen.



Lane	Sample	Harvest Mass (mg)
1	Ladder	N/A
2	Reference Lot 1	10
3	Reference Lot 1	5
4	Reference Lot 2	10
5	Reference Lot 2	5
6	Protein A Load	10
7	Protein A Load	5
8	PrismA Eluate	10
9	PrismA Eluate	5
10	Rapid A Eluate	10
11	Rapid A Eluate	5
12	Ladder	N/A

Figure 5: SDS-PAGE showing comparable purity between Sartobind® Rapid A and MabSelect PrismA™

5. Conclusion

Sartobind Rapid A provided enhanced yield, productivity, and capacity compared to MabSelect PrismA for purification of a large 545kDa Fc-fusion protein. Continued analytics will be completed to demonstrate that critical quality attributes (CQAs) are not impacted.

References: 1. Grünberg et al. (2022). Membranes; 2. App Note Sartorius Stedim Biotech: Sartobind® Rapid A as an Efficient, Cost-Effective Alternative to Traditional Protein A Resin Chromatography, August 2024; 3. App Note Sartorius Stedim Biotech: Sartobind® Rapid A: Robust mAb Capture at High Productivity, June 2022