Purolite® Life Sciences

Purpose-designed Protein A resins for Improved Downstream Process and Cost Efficiency in MAb purification

Authors: Patrick Gilbert, Hans Berg, Mark Hicks, Hans J. Johansson*, Caroline Tinsley and Duncan Sinclair
Purolite, Unit D, Llantrisant Business Park, Llantrisant, South Wales, CF72 8LF, United Kingdom
*Hans J. Johansson is the presenting author: Email: Hans.Johansson@purolite.com
www.purolitelifesciences.com

Summary and Conclusions

Figure 1 – ProcessReady 20 *Praesto* AC column

- Purolite has developed two new Protein A resins for cost effective MAb purification.
- The use of a classic MAb platform process with established resins, techniques and operational modes saves process optimization time
- Pre-packed ProcessReady columns with *Praesto* affinity and ion exchange resins eliminate non-value adding steps, remove risks and reduce costs

MAb Platform Processing

The chromatographic steps in a classic MAb platform process are a capture step with Protein A, followed by polishing steps using cation and anion exchangers. Key impurities to be removed are host-cell proteins (HCPs), DNA, aggregates, fragments and leaked Protein A ligand.

We recommend the classic 3-step process shown in Figure 2 as a proven and efficient approach. The use of a platform with established resins, techniques and operational modes saves process optimization time in the drive to produce material for early clinical trials in a speedy yet dependable manner.

Cation and anion exchangers have been used in either order in MAb polishing steps, and in both bind-elute (BE) and flow-through (FT) modes. The use of cation exchangers in BE mode allows fine tuning to secure maximal aggregate and HCP removal, while strong anion exchangers are commonly used as scavengers in FT mode to remove trace contaminants and ensure sufficient virus clearance. Using anion exchange in FT mode as step 2 can reduce conditioning requirements. The optimal order will be process dependent.

Figure 2 – Classic 3-step MAb platform process with *Praesto* resins

Production of early clinical phase material

Despite platform approaches to MAb processing, the production of materials for early-phase clinical trials can be costly. Next to skill- and labor-intensive PD work and the hands-on expertise required for operations, much of the expense comes from using the same purification materials that are used later for many cycles in full scale production. Particularly for more expensive resins like Protein A, the cost/cycle or cost/g product looks prohibitive when the resin is used for only a few cycles in clinicals production, instead of the 100s of cycles it is designed for.

Praesto AC is an example of a purpose-designed resin, designed and evaluated for production of early-phase clinical trial material.

The agarose development team

Purolite Ltd

Llantrisant Business Park
Llantrisant
Wales, UK
CF72 8LF
T: +44 1443 222336
F : +44 1443 227073
praesto@purolite.com

www.purolitelifesciences.com

A new alkaline stable Protein A resin – *Praesto* AP

Praesto AP is a new alkaline stable Protein A resin designed for large scale purification of monoclonal antibodies.

Figure 3 – Dynamic Binding Capacity at 10% breakthrough

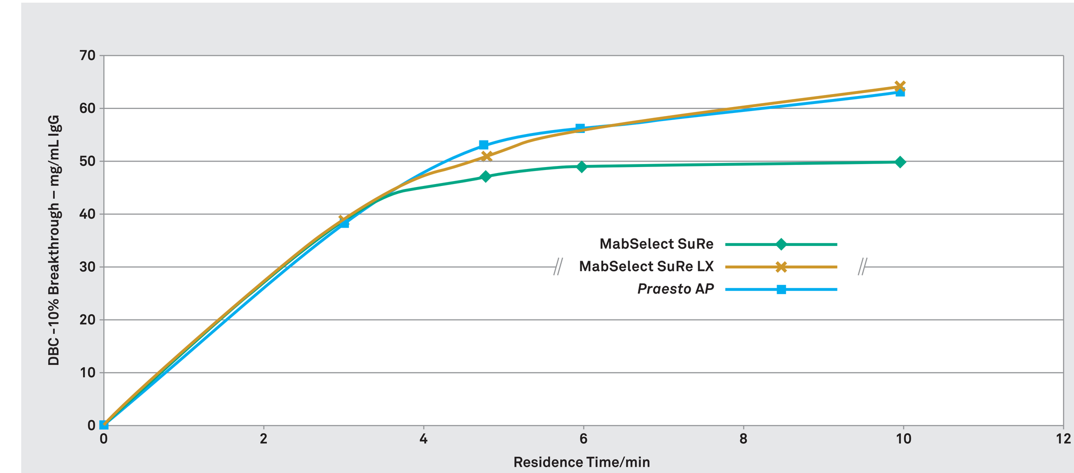

Figure 3 – Comparison of three Protein A resins. Dynamic binding capacity at 10 % breakthrough was measured as a function of residence time. The sample used was human polyclonal IgG at a concentration of 5.0 mg/mL in phosphate buffer, pH 7.4.

Figure 4 – Dynamic Binding Capacity after cleaning in place

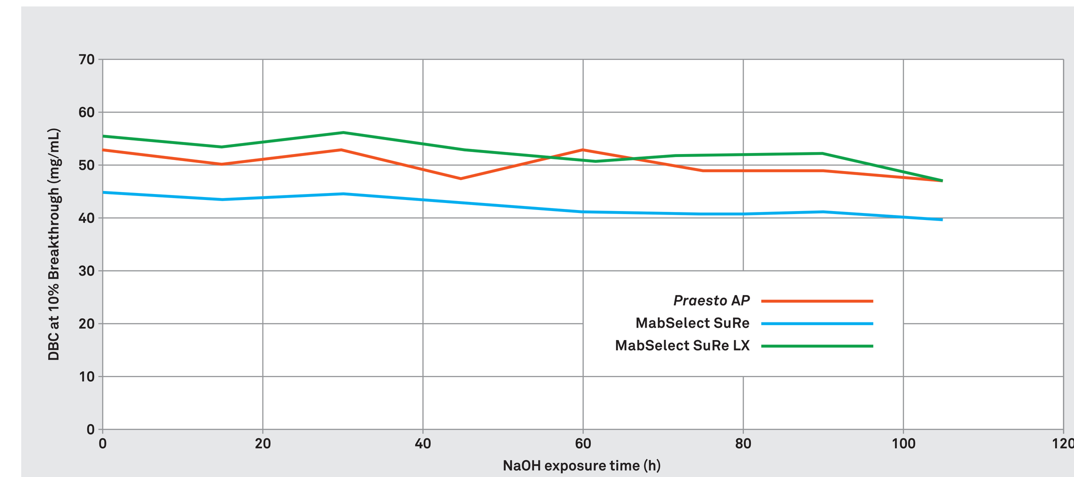

Figure 4 – Dynamic binding capacity as a function of sodium hydroxide exposure. DBC was measured at the start and at 15 hour incubation intervals at a residence time of 4.8 minutes. 0.1 sodium hydroxide solutions at a temperature of 22 C were used.

Praesto AC – For clinical manufacturing

Figure 5 – Dynamic Binding Capacity at 10% breakthrough

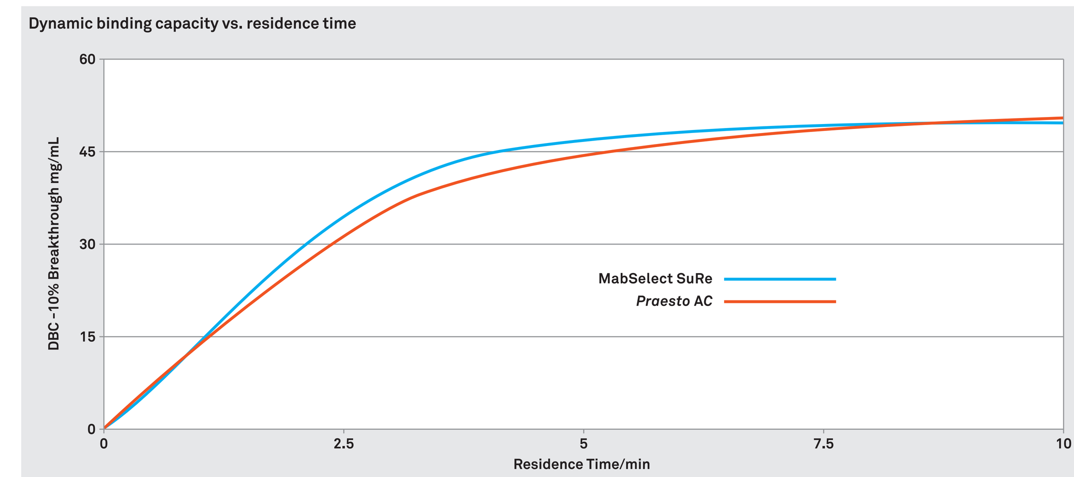

Figure 5 – DBC at various residence times measured with a 5 mg hIgG/mL solution, pH 7.4

Figure 6 – Sodium Hydroxide CIP Stability

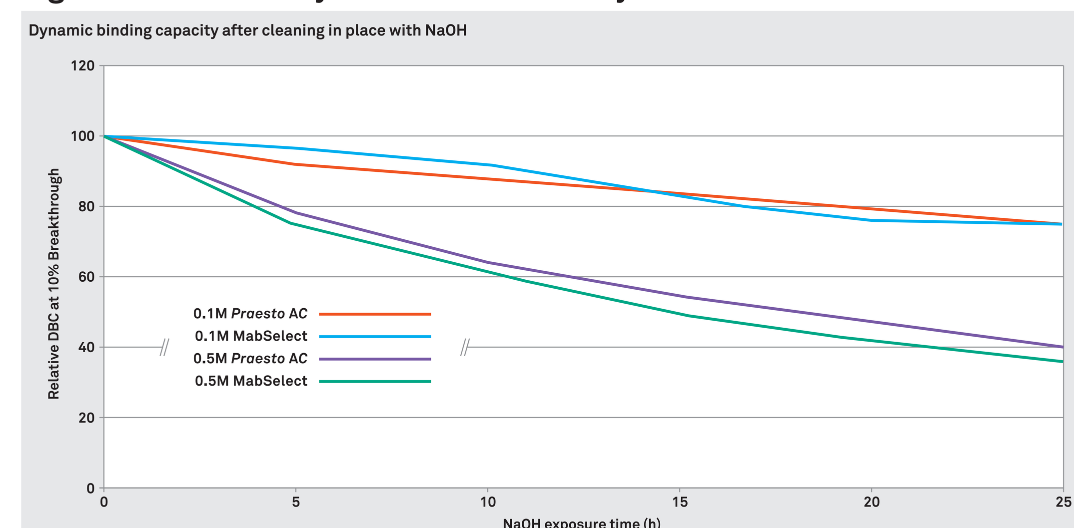

Figure 6 – CIP study over 25 hour exposure time using 0.1M and 0.5M NaOH. Five hours is equivalent to 20 cycles at 15 minutes contact time /cycle. DBC measured with a 5 mg hIgG/mL solution, pH 7.4.

Process and cost efficiencies

Time and cost savings can be substantial when purpose-designed resins and pre-packed formats are used. Table 1 compares MAb capture from a 1000L bioreactor at a 3 g/L titre, using *Praesto* AC or MabSelect SuRe in different pre-packed columns. Approximately 50% savings in resin costs can be achieved, without compromising buffer consumption or processing time. Particularly in a fast-paced environment, quick turnaround and optimized facility utilization are essential.

Table 1 – Process comparison: buffer consumption, processing time and cost of a MAb capture step

Process Example:	ProcessReady 20 *Praesto™* AC	ProcessReady 30 *Praesto™* AC	MabSelect SuRe 10 L RTP
Total Batch size (kg)	3	3	3
Column Diameter (cm)	20	30	25.1
Column Volume (L)	6.3	14.1	10
# of Cycles	14	6	8
Capacity	36	36	38.5
Pool Volume (L)	264	253.8	240
Buffer Volume (L)	1499	1438	1360
Total Processing (h)	19.1	8.1	10.0
Column Cost ($)	48,000	105,000	203,500

50% resin saving vs. GE

Table 1 – Assumptions: 1000 L bioreactor at 3 g/l (3kg batch), 17 CV per run at 300 cm/h, load at 260 cm/h, 80% of DBC at 4.8 minutes. RTP = ReadyToProcess.

Ion exchange resins for MAb purification*

Cation exchangers are present in most antibody processes with the primary function to remove product variants such as high molecular weight (HMW) aggregates and fragments. Here we compare four different cation exchangers with respect to DBC and removal of high molecular weight aggregates and host cell proteins. The generic conditions used were the same for all four resins and not individually optimized for each cation exchange resin.

Figure 7 – Dynamic binding capacity at 5 % breakthrough

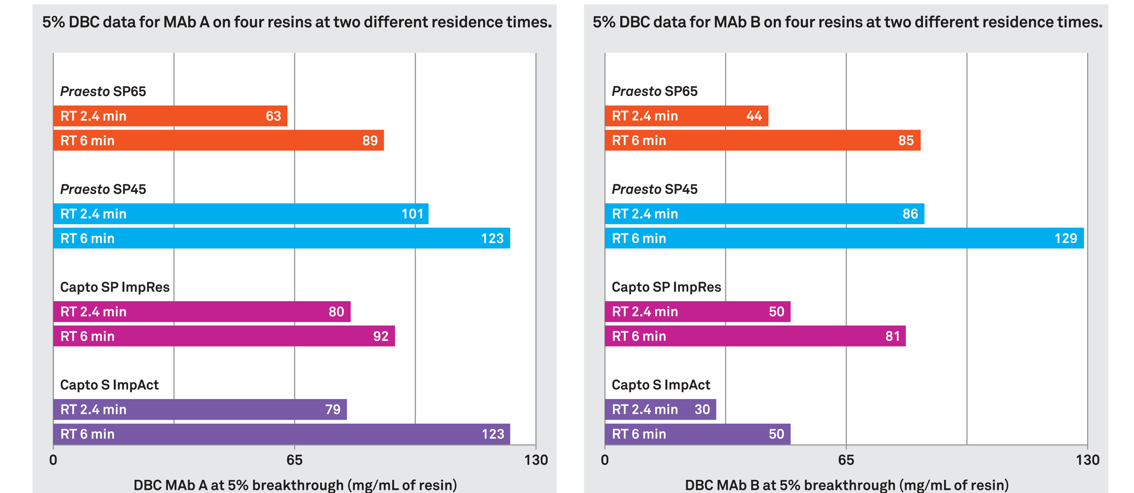

Figure 7 – DBC data for two different monoclonal antibodies of subclass IgG_1. MAb A: Protein A eluate adjusted to pH 5.5 with 1.75 M acetic acid. MAb B: 20 mM acetate, pH 5.0.

Figure 8 – HMW aggregate removal for MAb A

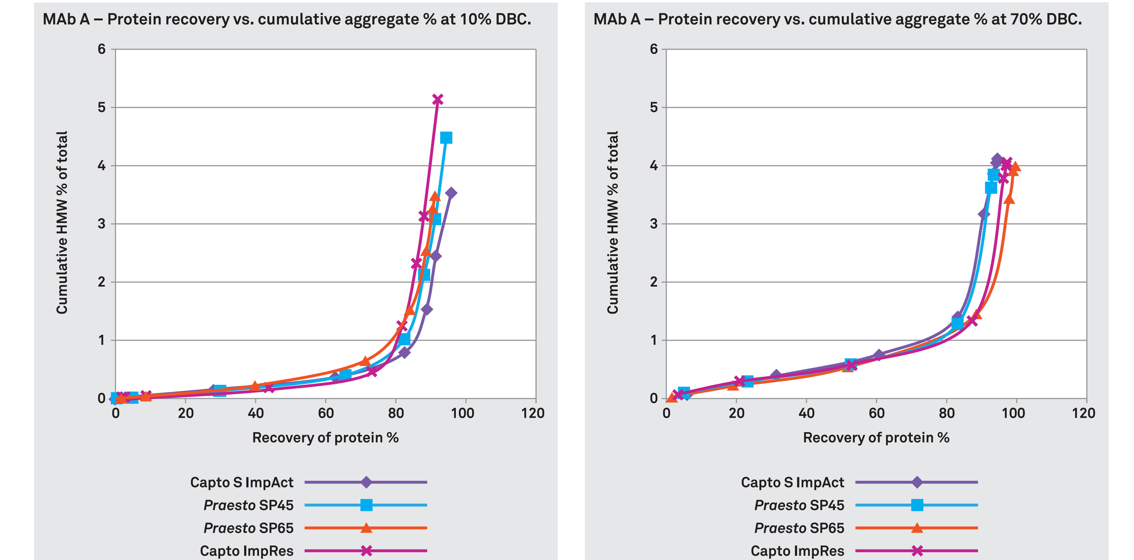

Figure 8 – Protein recovery vs. cumulative HMW aggregate removal at 10 and 70 % of the determined DBC. Elution with linear gradient over 20 column volumes from 20 mM sodium acetate, pH 5.0 to 20 mM acetate + 0.5 M sodium chloride, pH 5.0. The starting material (Protein A purified MAB) had a HMW aggregate content of 4 %.

Figure 9 – Host Cell Protein (HCP) clearance for MAb A

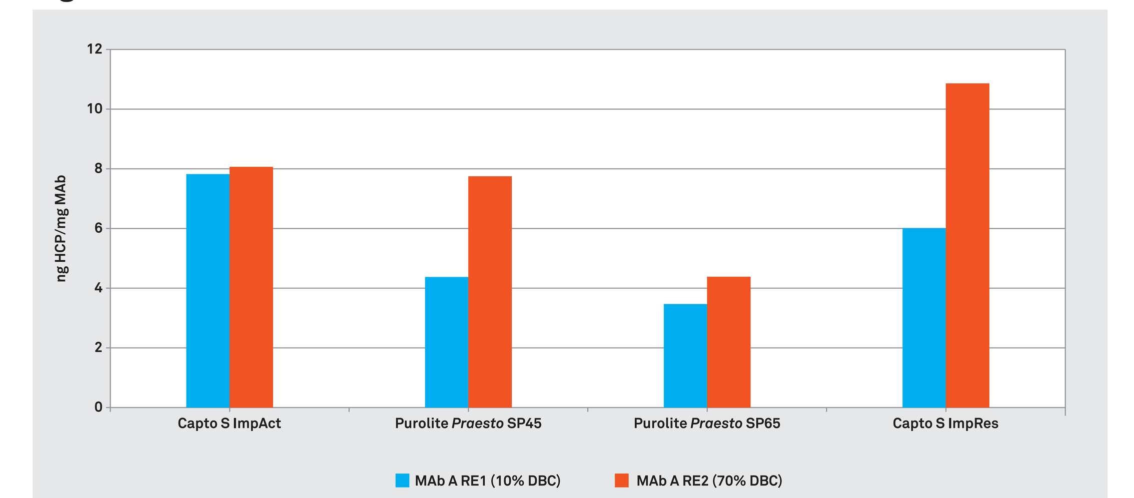

Figure 9 – There were no major difference in the HCP clearance ability under the conditions used. The Protein A purified starting material had a HCP content of approximately 40 ng HCP/mg MAb.

Praesto Q65 for polishing of MAb A at a load of 500 g/L resin

Strong anion exchangers are commonly used as a scavenger step in flow through mode to remove trace contaminants and ensure sufficient virus clearance. In this study we looked at the removal of host cell proteins under non-binding conditions on *Praesto* Q65. All fraction showed HCP levels of 1 ppm or lower.

Table 2 – *Praesto* Q65

Fractions	Rt 2.4 minutes	Rt 6.0 minutes
	(ng/mL)/mg/mL	(ng/mL)/mg/mL
Load	39.0	39.0
F1	0.44	0.25
F2	0.51	0.74
F3	0.70	0.75
F4	0.70	0.91
F5	0.96	0.66
F6	0.76	1.12
F7	0.99	1.07
F8	1.30	Below LOD

Table 2 – Conditions used: Approximately 500 mg MAb A/mL resin was loaded under non-binding conditions (Protein A eluate pH adjusted to 7.7) at two different residence times. 2.4 and 6.0 minutes. The column bed height was 6 cm.

*The monoclonal antibody purification evaluation was performed by Professor Anurag Rathore at the Indian Institute of Technology, Delhi.

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