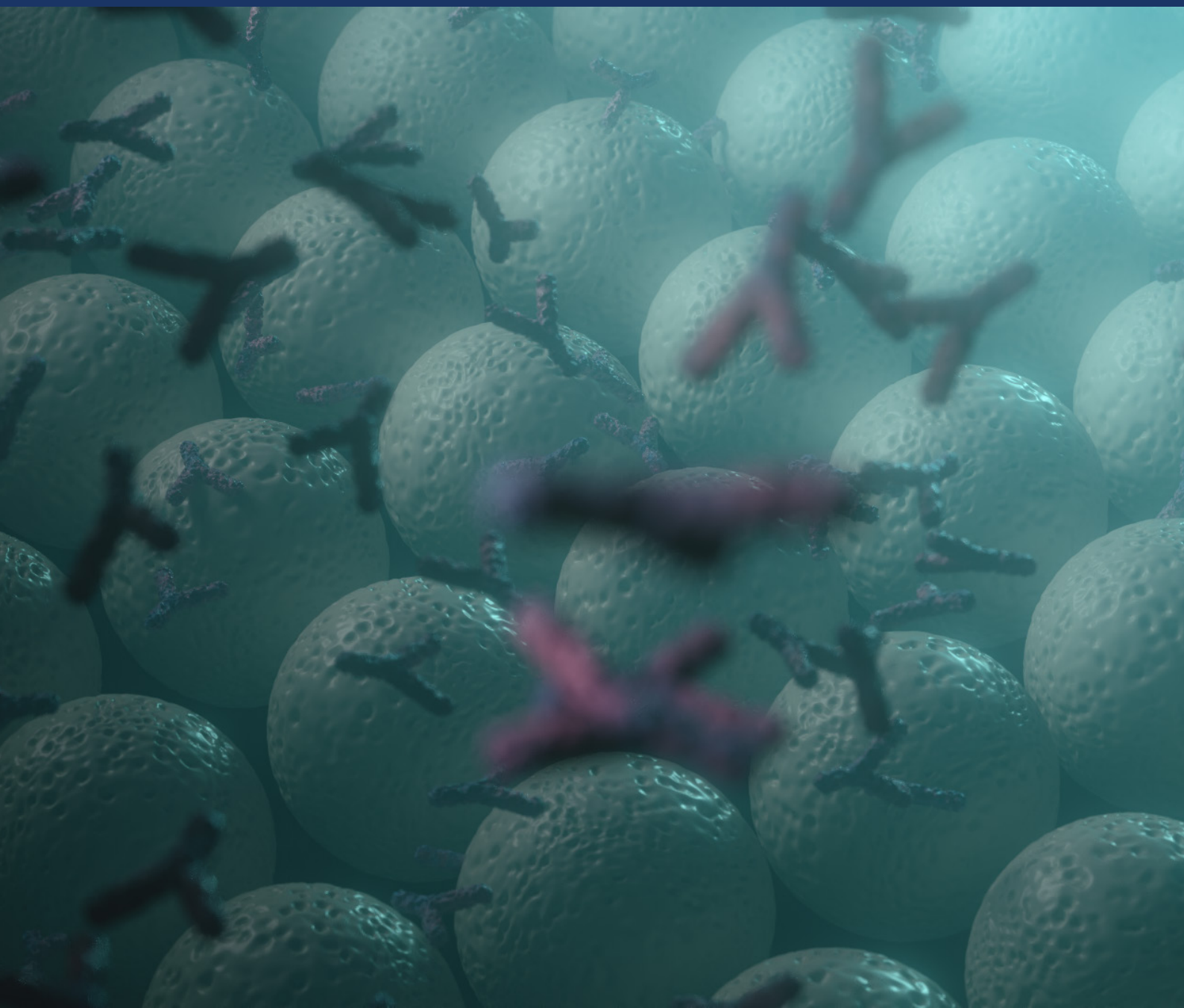
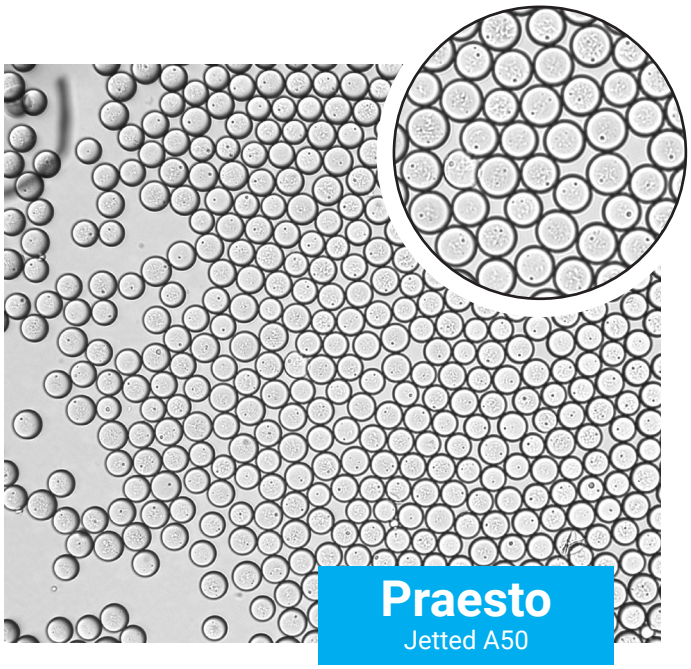
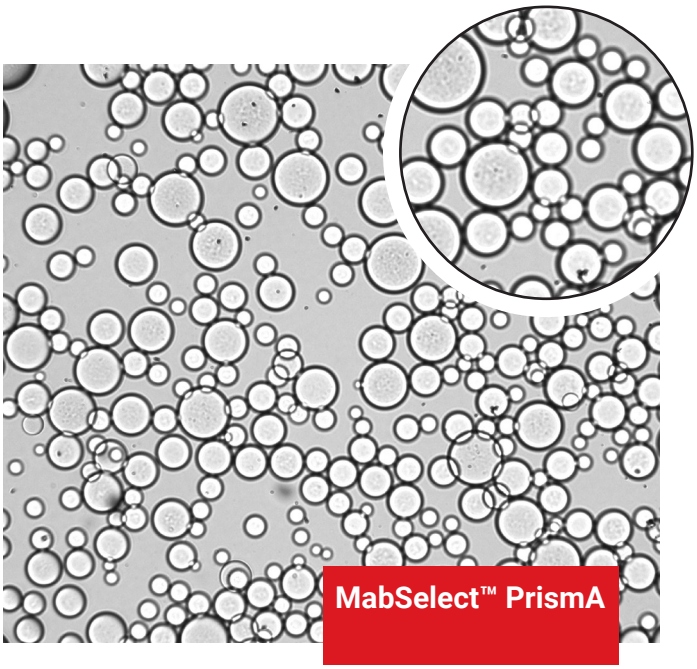
Purolite®

Novel Praesto® Jetted A50 HipH

Designed for purification of pH sensitive antibodies







Praesto® Jetting technology creates enhanced Protein A agarose resins for bioprocessing of recombinant proteins and monoclonal antibodies (mAbs)

What is Jetting?

Most agarose-based resins on the market are produced using Batch Emulsification technology, first utilized around 50 years ago. This produces a relatively wide particle size distribution.

'Jetting' is a Purolite®-patented, continuous manufacturing process for producing chromatographic resin beads with a narrow, uniform particle size distribution. Praesto® bioprocessing resins are the only agarose resins available with uniform particle sizes.

It is a more streamlined, efficient manufacturing method, which eliminates the need for the time-consuming screening step required in batch emulsification. This reduces manufacturing time in facilities, reducing lead times considerably.

Advantages of a narrow particle size distribution

Having a narrow particle size distribution is an advantage because it offers improved performance characteristics and more reproducible results. In simple terms, the more uniform the resin beads, the more consistently the resin will perform throughout scale-up.

In addition to uniform particle sizes, jetting technology eliminates fine particles in your resin.

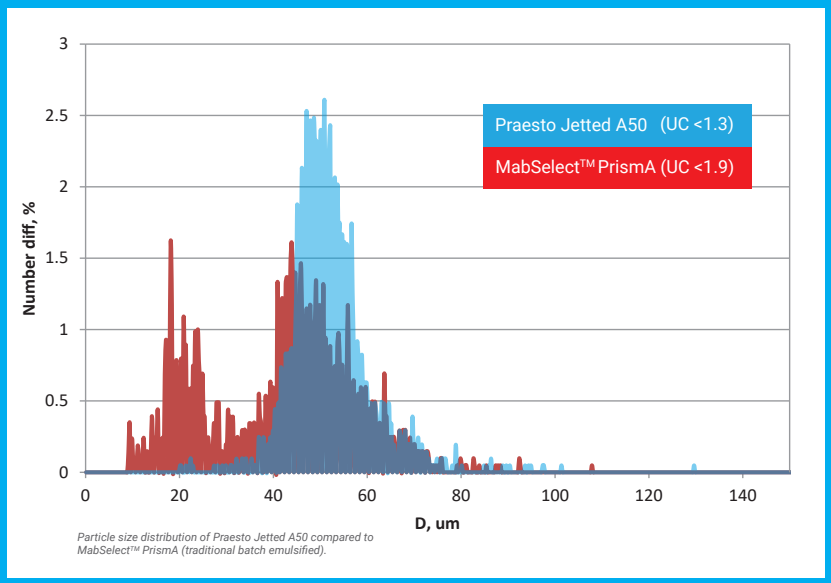
This enables you to:

- **Shorten your processing time**
- **Improve packing efficiency**
- **Improve lot-to-lot reproducibility**
- **Reduce your buffer consumption**
- **Improve cost efficiencies by up to 80%**

The process is also more environmentally friendly, as it utilizes no solvents and reduces agarose wastage, which can be as high as 40% in traditional manufacturing processes.

Upon request, we can also create custom-designed resin beads of almost any size. Biopharmaceutical manufacturers are able to work hand-in-hand with our R&D and applications scientists to tailor Protein A or ion exchange resin beads to a specific process or molecule.

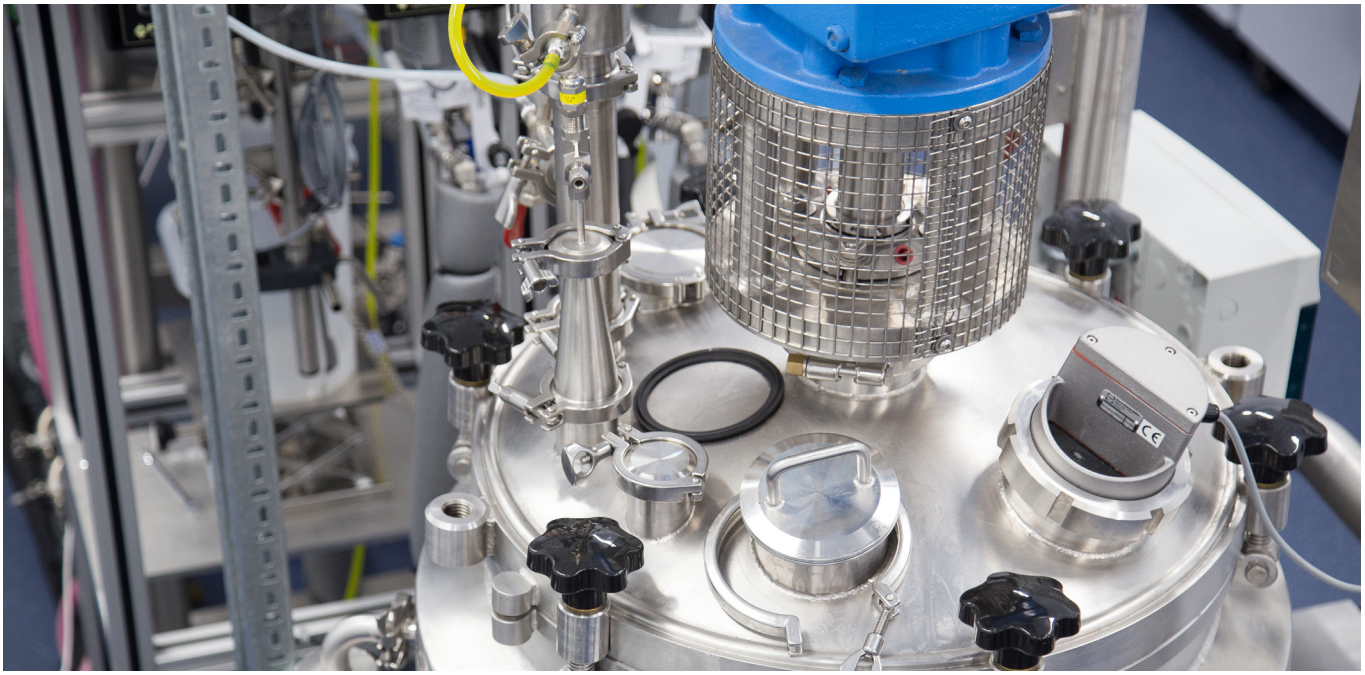
Figure 1 Comparing the number distribution between jetting and batch emulsification



Particle size distribution of Praesto Jetted A50 compared to MabSelect™ PrismA (traditional batch emulsified).

Key Data

After comparing the performance of Jetted A50 against the market standard, results show that Jetting technology produces a much tighter distribution of particles (UC < 1.3) than traditional batch wise emulsification technologies (UC < 1.9). The time-consuming screening step found in batch-wise emulsification is not required in the Jetting process, greatly reducing lead times.



The Praesto® range of protein A resins offers modern, high-flow affinity chromatography resins designed to deliver exceptional results in the recovery of active proteins.

These resins are available in a variety of formats to suit a variety of process needs. From high-throughput to commercial-scale manufacture, your process development and up-scaling can be further streamlined by using our pre-packed options. Building on established technology, Purolite in conjunction with Repligen have designed a new high pH eluting protein A resin.

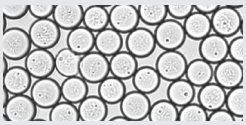
Commonly, a biopharmaceutical downstream purification processes employs a protein A chromatography resin as the capture step after clarification due to its high selectivity. Recovery of the target molecule is achieved by lowering the pH to 3-3.5. However, for several applications, the low pH provides stability issues with the target molecule. In these applications, other less selective techniques such as cation exchange of hydrophobic interaction are selected for the capture step.

About Praesto Jetted A50 HipH

The new Praesto Jetted HipH A50 allow users to gain the benefits of the high selectivity that protein A offers without the low pH instability of their target molecules.

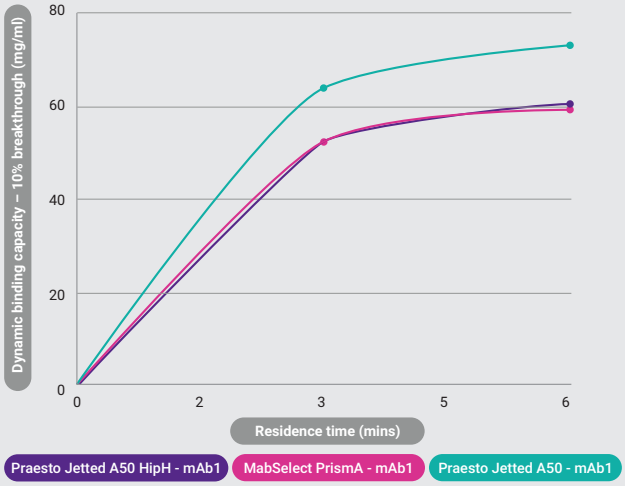
The unique elution properties of Praesto Jetted A50 HipH can offer significant improvements in associated process impurity removal during modern monoclonal antibody purification.

Table 1 **Resin characteristics or Praesto Jetted A50 HipH.**

Polymer Structure	Highly cross-linked agarose
Dynamic binding capacity	Up to ~60 mg hIgG/ml resin
Average particle size	50 µm
Particle size range	95% between 35 – 90 µm
Pressure/Flow specifications	Up to 250 cm/h (20 x 60 cm column)
pH stability (Working range)	3 – 12
pH stability (CIP)	2 – 14
Recommended storage	2 – 8°C in 20% ethanol
Proprietary uniform bead structure	

Performance data

Capacity – mAb1



Capacity - hIgG

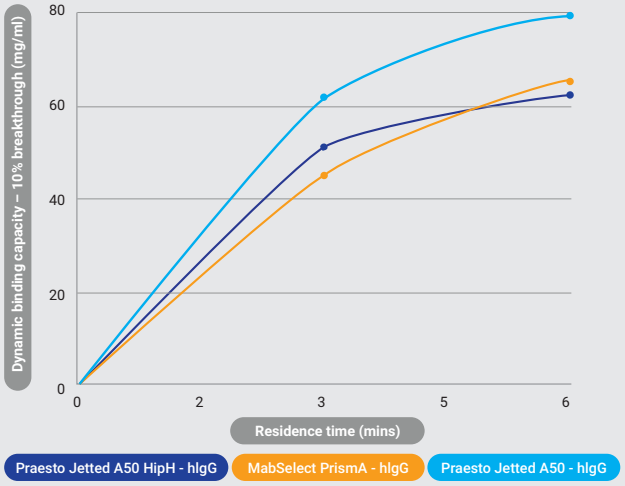


Figure 2a and 2b Breakthrough capacity for polyclonal immunoglobulin G (hIgG) and an IgG1 monoclonal antibody (mAb 1) of Praesto Jetted A50 HipH, Praesto Jetted A50 and MabSelect SuRe LX.

Praesto Jetted HipH A exhibits excellent capacity for polyclonal human immunoglobulin G and an IgG1 subclass monoclonal antibody. Excellent capacity and selectivity are available for molecules previously sensitive to low pH elution. An equivalent capacity to the market leading protein A resin is achievable using Praesto Jetted HipH A.

Alkaline Stability

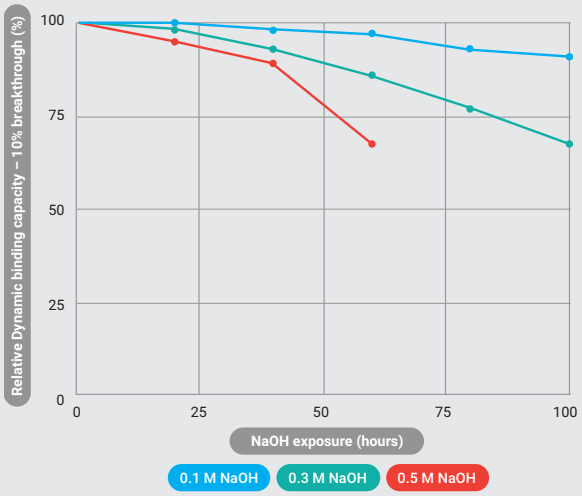


Figure 3 Sodium hydroxide stability of Praesto Jetted A50 HipH. Tested with hIgG at selected exposure intervals.

Sodium hydroxide (NaOH) is commonly used in bioprocessing as an industry standard for cleaning in place, as such it is increasingly important for capture resins to be extremely alkaline stable. Sodium hydroxide exhibits high efficiency in removing bound proteins, nucleic acids, and lipids from bioprocess resins, alleviating the risk of fouling on heavily burdened protein A columns. Praesto Jetted HipH A50 has been shown to be alkaline stable for over 100 hours exposure to 0.1 M NaOH.

Process Flow

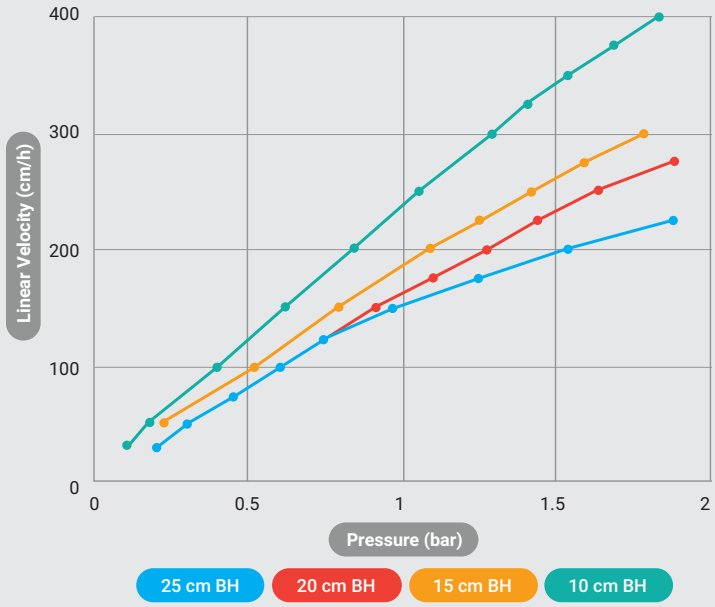


Figure 4 Pressure flow curve for Praesto Jetted A50 HipH packed in a BPG 300 column at 25, 20, 15 & 10 cm bed height. Tested using demineralised water at 20 °C

The uniform particle size and highly cross-linked agarose base bead of Praesto Jetted HipH A50 elucidates the benefit of increased surface area of a smaller bead size without severely impacting the pressure flow properties. Praesto Jetted A50 has good flow properties, with flows of up to 250 cm/h achievable in a standard mAb platform process,

Process performance

Purification performance of Praesto Jetted A50 HipH using elution conditions from acidic to mild was investigated. Quantitative elution was achieved up to pH 5.0 using a clarified harvest of an IgG1 subclass monoclonal antibody with recovery levels greater than 95% and elution within 2.5 column volumes. Leached Protein A levels determined to be similar to that of Praesto Jetted A50.

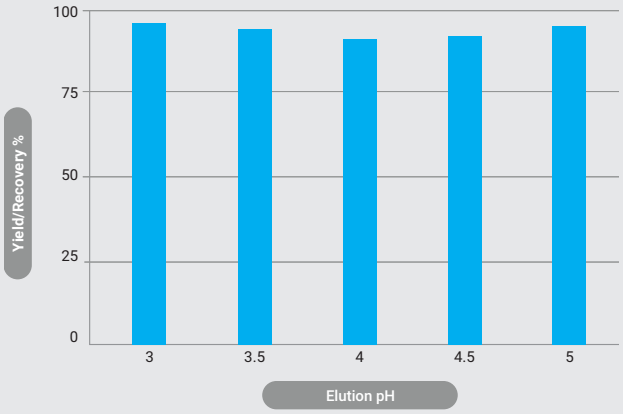


Figure 5 Recovery/Yield from protein A capture of an IgG1 clarified harvest with elution at pH 3 - 5 tested.

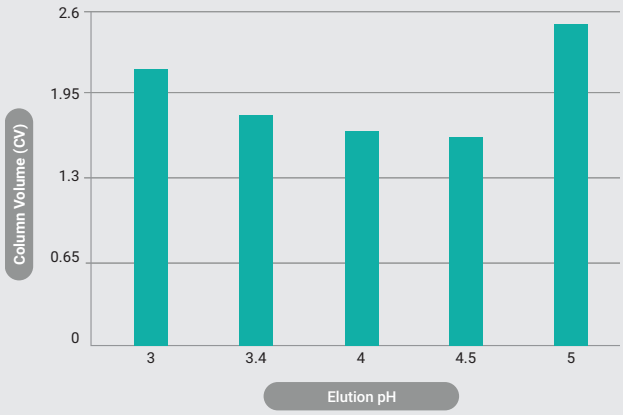


Figure 6 Peak elution column volumes from protein A capture of an IgG1 clarified harvest with elution at pH 3 - 5 tested.

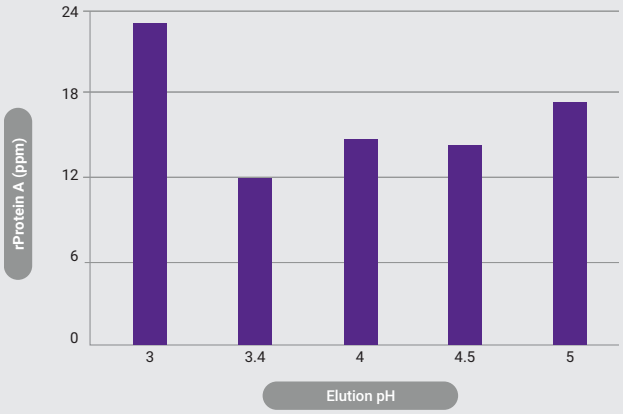


Figure 7 Leached protein A levels from protein A capture of an IgG1 clarified harvest with elution at pH 3 - 5 tested.

Process performance - Customer Evaluation

Customer evaluations have shown the effect of using the unique properties of Praesto Jetted A50 HipH to elute at much higher pH when compared to conventional protein A chromatography. Two mAbs were tested, listed as mAb 2 and mAb 3.

Minimal impact on purity is observed by increasing the elution pH of the buffer to elute mAb 2 and mAb 3. The log reduction in host cell proteins and separation of the target molecule from residual DNA is markedly improved by eluting at a higher pH.

Table 2: pH elution and eluate conditions for mAb 2 using Praesto Jetted A50 HipH.

pH of elution buffer	Elution peak CV	pH of eluate
pH 3.5	1.68	4.46
pH 3.8	1.76	4.57
pH 4.0	1.80	4.71
pH 4.2	1.80	4.86
pH 4.5	1.76	5.10
pH 4.8	4.04	5.13
pH 5.0	5.32	5.26

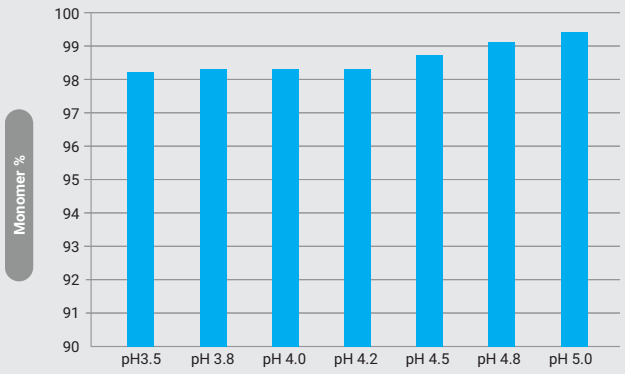


Figure 8 Purity of mAb 2 after protein capture using Praesto Jetted A50 HipH with elution buffer pH from 3.5 to 5.0.

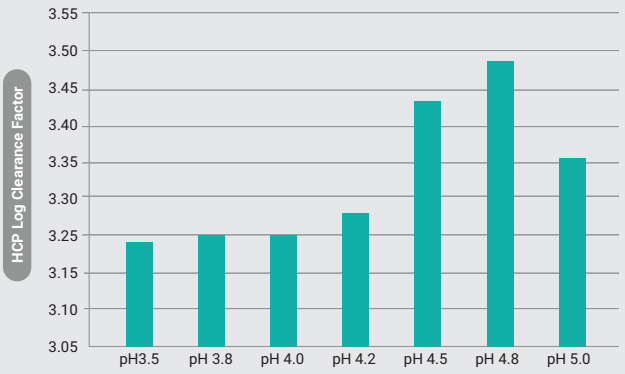


Figure 9 Log reduction of host cell protein levels associated with mAb 2 after protein capture using Praesto Jetted A50 HipH with elution buffer pH from 3.5 to 5.0.

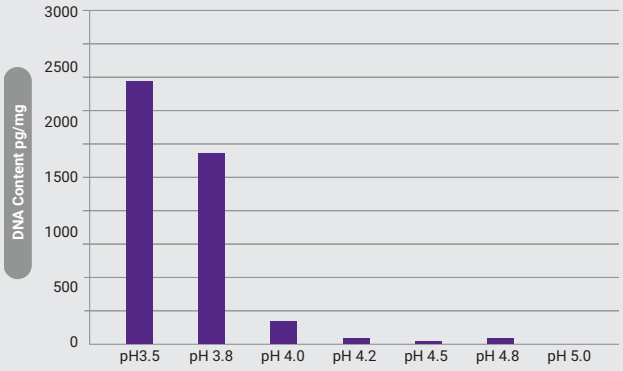


Figure 10 Residual DNA levels associated with mAb 2 after protein capture using Praesto Jetted A50 HipH with elution buffer pH from 3.5 to 5.0.

Table 3 pH elution and eluate conditions for mAb 3 using Praesto Jetted A50 HipH

Elution pH	Elution CV	Elute pH
pH 3.5	1.44	4.46
pH 3.8	1.44	4.60
pH 4.0	1.32	4.71
pH 4.2	1.48	4.80
pH 4.5	1.48	5.02
pH 4.8	1.68	5.21
pH 5.0	1.68	5.36

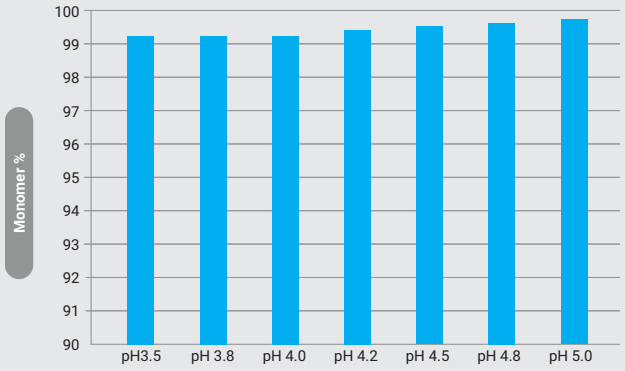


Figure 11 Purity of mAb 3 after protein capture using Praesto Jetted A50 HipH with elution buffer pH from 3.5 to 5.0.

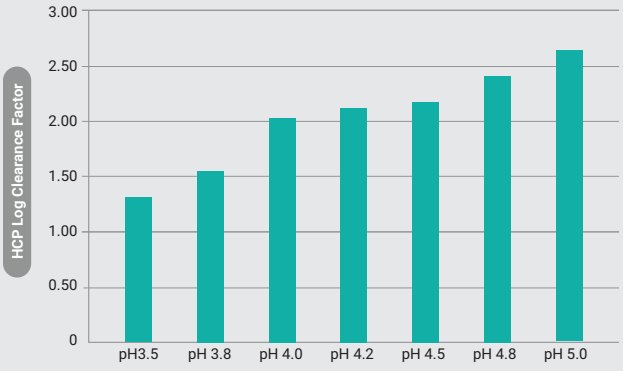


Figure 12 Log reduction of host cell protein levels associated with mAb 3 after protein capture using Praesto Jetted A50 HipH with elution buffer pH from 3.5 to 5.0.

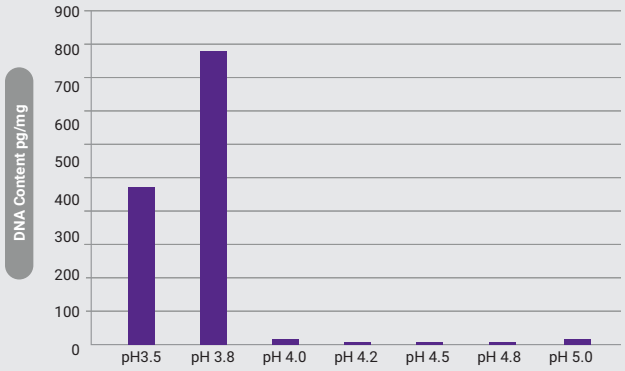


Figure 13 Residual DNA levels associated with mAb 3 after protein capture using Praesto Jetted A50 HipH with elution buffer pH from 3.5 to 5.0.

Summary

In partnership with Repligen, Praesto Jetted A50 HipH is Purolite's latest technological innovation in affinity chromatography. The new protein A resin addresses the limitation of pH sensitive molecules to use conventional protein A chromatography where low pH elution is required. The selectivity of protein A is now available to those sensitive molecules.

Significant improvements can be gained with associated process impurity removal in conventional monoclonal antibody purification by utilising the unique elution properties of Praesto Jetted A50 HipH.

Security of Supply

Despite the global pandemic causing supply challenges across the global to many industries and markets, including the biopharmaceutical sector where the supply of process critical raw materials became particularly effected, delaying clinical trials. The pandemic has taught the biopharmaceutical industry to consider the entire process from start to finish and highlight key process dependent raw materials. This exercise once completed identified in particular process critical singly sourced items. In the case of the production of monoclonal antibodies, chromatography resins in particular Protein A resins are highlighted. Depending upon the risk classification of the raw materials, process owners work to implement strategies that mitigates the future supply risk. Today, it is common practice to understanding your suppliers' capabilities to manufacture including their own supply chain and associated key high-risk items.

The ultimate goal of a responsible supplier whether that is raw materials, or the final drug product is to ensure the uninterrupted supply.

Supply and demand challenges.

In supply/partnership discussions within the BioPhorum's Supply Partner Phorum around the surety of supply, discussions involving suppliers and biomanufacturers it is clear that demand is growing and with-it surety of supply pressure for the supply of Protein A to the market. As a result of these discussions, Purolite considers the current security of supply of protein A resins in the industry to need strengthening to ensure uninterrupted supply. Having listened to the needs of the market and key customers, Purolite has taken the strategic decision to improve security of supply and has invested heavily into our manufacturing capabilities that will provide faster supply of bioprocessing resins to the industry.

How has this been achieved?

This investment gives Purolite the capability to meet 100% of the global demand for bioprocess chromatography resins. The increased output will come from a second dedicated agarose manufacturing facility located in Pennsylvania, USA coming on-line end of 2021. For biopharmaceutical producers located in the USA, this new facility ensures they are not dependent upon imports from outside the country. This new high value state of the art manufacturing facility, alongside the current facility located in the United Kingdom, makes Purolite the first bioprocess resin supplier to have manufacturing sites on two continents with the ability to flex as required output across two independent locations. This approach sees a new advent of Security of Supply, version 2.0.

'The Pandemic has highlighted that for the biopharmaceutical manufacturing industry security of supply and supply chain resilience is best served when major suppliers are able to manufacturer the same products to the same quality standards in multiple regions of the world.' Bob Brooks, Supply Partner Phorum Lead, BioPhorum