

The Impact of Elution pH on Product Quality of Fc-Containing Proteins



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The antibody purification industry is being challenged by the development of a wider array of molecules containing an Fc-region. There is a large diversity in the Fc-containing molecules, but also in the cell culture feeds that produce them. This diversity is a challenge, but also an opportunity for the resin manufacturing industry to innovate and evolve. Protein A affinity chromatography is the most widely employed technique to purify Fc-containing proteins and antibodies due to its high specificity. While the elution of the target molecule is triggered by lowering the pH up to 3–3.5, this does not always create the optimal conditions for the target molecule or to remove impurities (aggregates, host cell proteins (HCP), DNA, and viruses).

Praesto™ Jetted A50 HipH

Praesto Jetted A50 HipH has been designed to address challenges in eluting pH-sensitive mAbs and Fc-containing proteins, which can become unstable at pH levels typically used for elution with other Protein A resins. This resin provides alkaline stability, capacity of 60 g/L for polyclonal human IgG, and elution up to pH 5. Recent reports using salt suggest elution is possible at even higher pH for some molecules (YiFeng Li et al, Protein Expression and Purification 229 (2025) 106677).

Herein, we present the benefits that can be achieved with acid labile molecules, overall impurities, and viral clearance using a milder elution pH with Praesto Jetted A50 HipH.

Figures 1 shows the purification of an IgG1-based mAb using six different protein A resins. The purification was repeated five times, each time changing the pH of elution. As expected, recovery decreased with increasing pH for standard protein A resins. Praesto Jetted A50 HipH parentheses around maintains a high yield over each pH tested. The study was further expanded to look at additional molecules, two of which are shown in Figure 2. This study was carried out using IMCS tips containing 50 µl of the stated resin.

Figures 4, 5, 6, and 7 evaluated three molecules: an IgG1 Fc-fusion protein (Fc-Fusion), an IgG4 monoclonal antibody (mAb), and an IgG1 bispecific antibody (BsAb). All experiments were performed at a 6-minute residence time using a 45 g/L loading. An intermediate wash of 50 mM Tris-HAc, 0.5 M at pH 7 was used for Praesto Jetted A50 HipH and 50 mM Tris-HAc, 0.5 M at pH 5.5 for Praesto Jetted A50, Competitor A, and Competitor B. Elution pH for Praesto Jetted A50 HipH was pH 4.5 for Fc-fusion and mAb, with an elution pH of 4.8 for BsAb. Elution pH for Praesto Jetted A50, Competitor A, and Competitor B for all three molecules was 3.5.

Eluting at a higher pH helps prevent aggregates, reduces HCP and DNA, and improves viral clearance. The performance of Praesto Jetted A50 HipH, was compared to Praesto Jetted A50 and a competitor (B) resin for viral clearance. The molecule eluted at pH 3.5 from all resins tested, but only at pH 4.5 from Praesto Jetted A50 HipH. Thus, all these conditions were assessed by spiking enveloped (Xenotropic Murine Leukemia Virus, MLV) and non-enveloped (Murine Minute Virus, MMV) viruses (Figures 8 and 9).

Finally, a bioprocessing resin must exhibit exceptional dynamic binding capacity and pressure flow characteristics, as demonstrated in Figures 3 and 10.

