Address Challenges of Bispecifics and Antibody Fragments with Novel Affinity Resins

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Protein A affinity chromatography remains the preferred method for purifying monoclonal antibodies (mAbs) because of its efficiency in capturing such proteins by binding to their Fc and variable heavy (VH3) regions. The bind–elute process used in such purification follows a straightforward workflow. First, a target mAb is captured by the immobilized ligand on a chromatography resin in a column during the binding step. Unbound feedstock and impurities are removed through a wash step, which is followed by elution of the bound mAb from the column by lowering the pH. This technique has proven to be effective, consistently delivering highly pure material.

Innovations in protein A affinity resins have enabled increases in purity and yield. One example is the MabCapture C affinity resin, which has an engineered protein A ligand that binds to the constant heavy (CH2–CH3) domain interface and the VH3 region of an antibody. Featuring high capacity and a highly crosslinked agarose backbone, this resin is designed specifically to improve the productivity and efficiency of antibody purification processes.

The Need for Advanced Purification Tools

Although traditional protein A capture is highly effective for many mAb applications, the distinct characteristics of next-generation antibodies — such as bispecifics (bsAbs), Fc-fusion proteins, and antibody-binding fragments (Fabs) — have created demand for other affinity resins. Such antibody modalities can lack or exhibit altered protein A binding sites and/or heightened pH sensitivity that makes them prone to increased aggregation, light-chain–heavy-chain mispairings, and the formation of light-chain dimers.

To meet those evolving needs, CaptureSelect technology is used to target different regions of the antibody or fragment to achieve desired specificities (Figure 1). The discovery process begins with screening multiple variable heavy-chain (VHH) affinity ligands. Then the final ligand is selected based on specific factors such as target specificity, milder elution condition, and base stability. The result is a resin with a ligand that has high affinity for a given target, which allows for high yield and purity in a single chromatography step. Mild elution conditions and the ability to reuse a resin provide additional advantages. For product safety, the ligands are produced by Saccharomyces cerevisiae in a process that is free of animal-origin materials.

CaptureSelect CH1-XL resin binds to the constant domain (CH1) of the antibody heavy chain and thus works for all mAbs and Fabs, regardless of light-chain type.

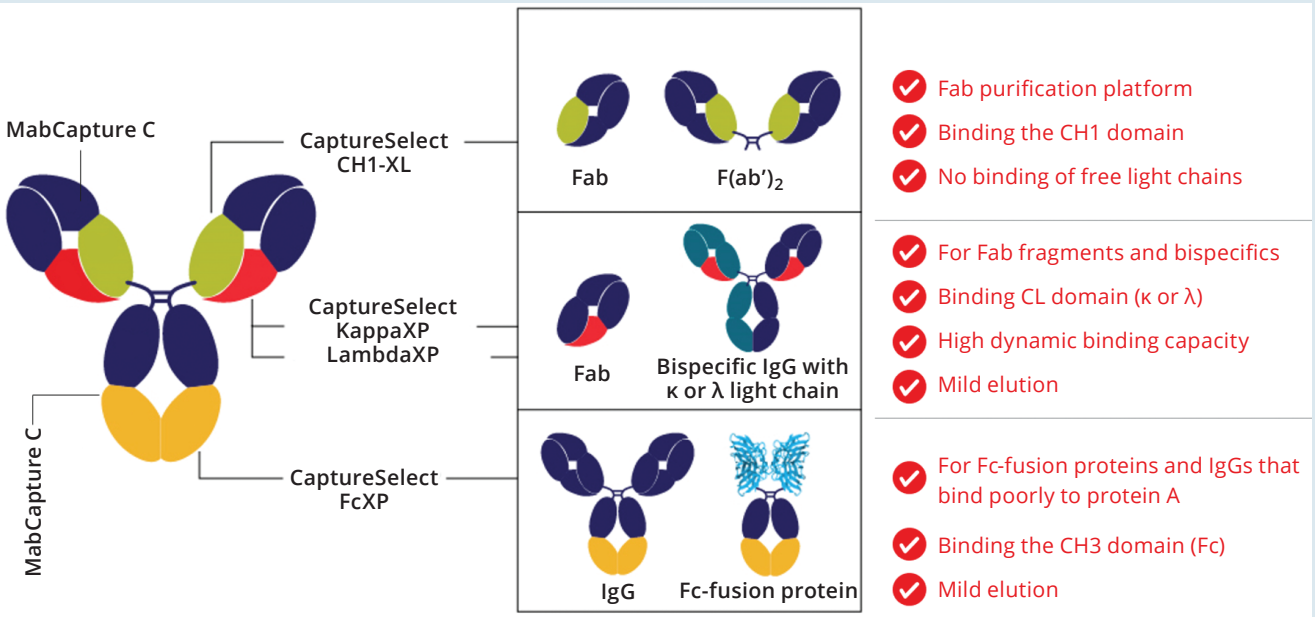
KappaXP and LambdaXP resins bind to the constant domains of the κ and λ light chains, respectively, helping to ensure 100% coverage of antibody fragments that contain either chain. Both resins work with mild elution conditions that are critical for labile antibodies, and recommended for bispecifics with κ and/or λ light chains.

CaptureSelect FcXP resin binds to the CH3 region on the Fc domain. This resin is optimized for immunoglobulin G (IgG) antibodies that bind poorly with protein A binding as well as Fc fusion proteins. For proteins that are prone to aggregation, it works with relatively mild elution conditions.

Capture of CH1-Containing Bispecifics

CaptureSelect CH1-XL resin binds to the CH1 domain of all human IgG subclasses, which is highly suitable for Fab-fragment purification because incorrectly assembled Fab fragments and free light chains will not bind. This chromatography resin has a high dynamic binding capacity (DBC) of

Figure 1: CaptureSelect resins are designed for affinity purification of novel mAbs and fragments; CH = constant heavy chain, CL = constant light chain, Fab = antibody-binding fragment, Fc = crystallizable fragment; IgG = immunoglobulin G.



about 19 mg/mL for polyclonal Fab fragments. Elution can be performed under mild conditions such as using 50 mM sodium acetate at pH 4.0–4.5.

Spooner et al. described the use of this resin in a one-step process for purification of Lucentis (ranibizumab), a correctly assembled Fab fragment (**1**). As determined by analytical size-exclusion chromatography (SEC), purity was 98% Fab. In a single affinity step, the recovery was 86%. In a subsequent experiment, samples were analyzed by sodium-dodecyl sulfate capillary electrophoresis (CE-SDS) for Fab and light-chain dimers. Two sample loads with different heavy- and light-chain ratios to generate different Fab and light-chain dimer ratios were 72% and 47% Fab. After capture on the CH1-XL resin, analysis of the elution fractions showed 100% enrichment of Fab in both loading parameters and no detectable light chain dimers.

The CaptureSelect CH1-XL resin also has been used for separation of an asymmetrical bsAb consisting of three chains: one heavy chain with a CH1 domain, one heavy chain with no CH1 domain, and a κ light chain. In addition to the active heterodimer, inactive homodimers, half-mAbs, and free light chains also were expressed. With conventional protein A affinity, the active and inactive species coeluted, and low-pH (3.0) elution resulted in 28% high–molecular-weight (HMW) aggregates.

The CaptureSelect CH1-XL resin specifically targeted the heavy chain with the CH1 domain. The inactive species did not bind to the column during loading and was detected in the flow-through. The milder elution at pH 4.0 reduced the presence of HMW species. SDS polyacrylamide gel electrophoresis (SDS-PAGE) showed that the CaptureSelect CH1-XL resin bound preferentially to the active bispecific antibody, whereas the protein A process yielded additional homodimer species, heavy chains, and half-mAbs (Figure 2, left panel). Analytical SEC confirmed that the CH1-XL elution pool contained 98% of the active bispecific — compared with 72% in the protein A elution pool (Figure 2, right panel). Milder elution conditions used with the CaptureSelect CH1-XL resin (50 mM acetic acid, 10% glycerol, 10% sucrose at pH 4.0) significantly reduced aggregation from 28% to 2% HMW.

Strong Aggregate Separation

High aggregation content is a common issue with bsAbs. Although protein A affinity chromatography is highly effective for initial product capture, it typically cannot separate aggregates. That task usually falls to polishing steps such as hydrophobic-interaction chromatography (HIC) or mixed-mode chromatography. However, relying solely on polishing steps for aggregate removal can compromise the robustness of a downstream process, especially when high aggregate content is a problem.

CaptureSelect FcXP resin binds to a single domain on the CH3 region of IgGs, whereas protein A engages with the CH2, CH3, and VH3 regions. The FcXP resin can be used with milder elution conditions (pH 4.0–4.5) than protein A, which is beneficial for IgG-fusion proteins or mAbs that cannot withstand low-pH elution. The CaptureSelect FcXP resin has a high DBC of >40 g/L.

While investigating the effect of mobile-phase additives on antibody elution with an aggregation-prone bsAb, Dong et al. observed a significant difference between the elution profiles of protein A and FcXP resin under a linear pH gradient (**2**). From the same load material, the CaptureSelect FcXP resin demonstrated a higher level of aggregate removal. Intrigued by those results, the authors further evaluated aggregate separation capabilities of CaptureSelect FcXP resin. They used a pH-gradient elution from 5.5 to 3.5 to compare the two affinity resins.

Using FcXP resin improved aggregate separation for the bsAb. In the protein A chromatogram (not shown), elution appeared as a single

peak with a slight divot at the top, and monomer content in the elution pool was 91% measured by analytical SEC. By contrast, the CaptureSelect FcXP chromatogram displayed two distinct elution peaks: the first representing monomer (with over 99% monomer content measured by analytical SEC) and the second shoulder peak corresponding to aggregated HMW species. Additionally, FcXP elution occurred slightly earlier than that from protein A, offering more flexibility in process development.

Single-Step Purification and Removal of Mispaired Species

CaptureSelect Kappa and Lambda XP resins are excellent for separating homomeric and heteromeric bsAbs by targeting their respective light chains. CaptureSelect Kappa XP specifically binds to the κ light chain and has a DBC of 20–30 g/mL for κ Fab and 30–40 g/L for IgG. Mild elution conditions can be used to preserve the monomeric state of bsAbs. CaptureSelect Lambda XP is designed to bind the λ light chain, with a DBC of about 35 g/L for IgG.

Another study describes the use of light-chain binding avidity to control for mispaired bispecifics during production of asymmetric bispecific antibodies (**3**). The study compared different light-chain resins with a bsAb construct containing both κ and λ light chains. The objective was to isolate correctly formed bsAbs using a single column to reduce cost and time rather than using separate κ and λ resins in two steps. The researchers screened a variety of affinity resins with a pH elution gradient to evaluate their separation potential and chose Kappa XP and Lambda XP resins because they demonstrated the greatest resolution of mispaired species.

The Kappa XP resin delivered an acceptable recovery rate of 70%, with bsAb purity reaching 100% as measured by analytical HIC; aggregation levels were <3% according to analytical SEC. Recovery was higher for the Lambda XP resin, at 86%, but product quality declined slightly, with purity at 95% and aggregation levels at 11%. Based on those results, the team performed additional evaluations using different modifiers to enhance recovery from the

Figure 2: CaptureSelect CH1-XL showed preferential binding to an active bispecific antibody (bsAb) (LEFT) and a higher percentage of active monomer content in the elution pool (RIGHT); SDS-PAGE = sodium-dodecyl sulfate polyacrylamide gel electrophoresis, LD = load, FT = flow-through, E = elution, MW = molecular weight, aSEC-HPLC = analytical size-exclusion high-performance liquid chromatography.

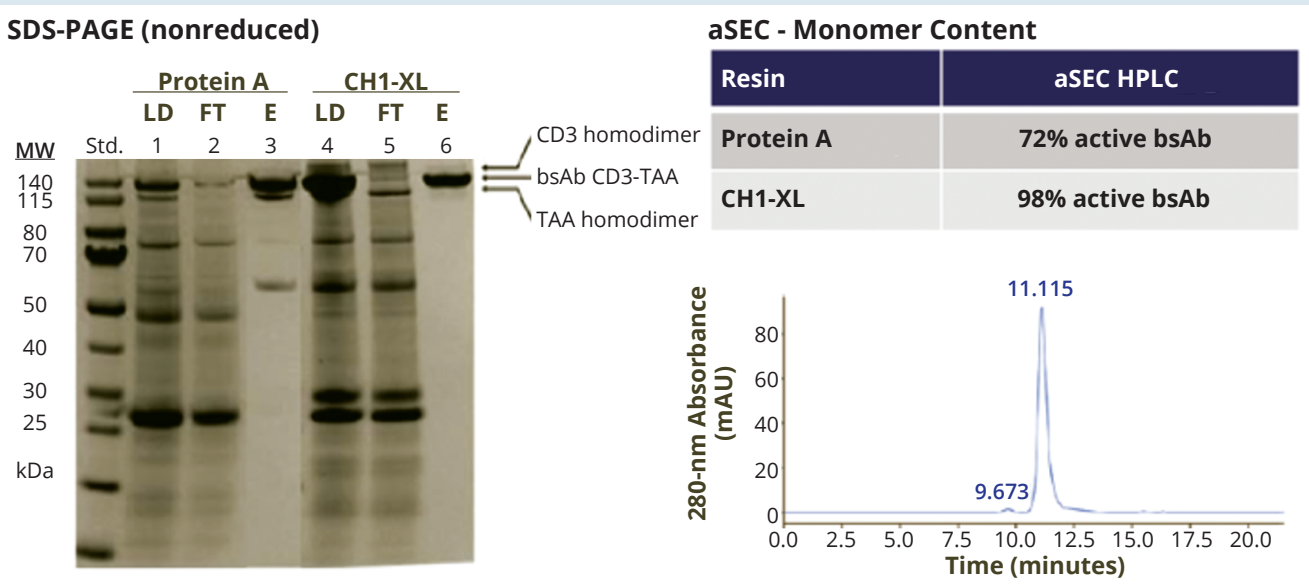
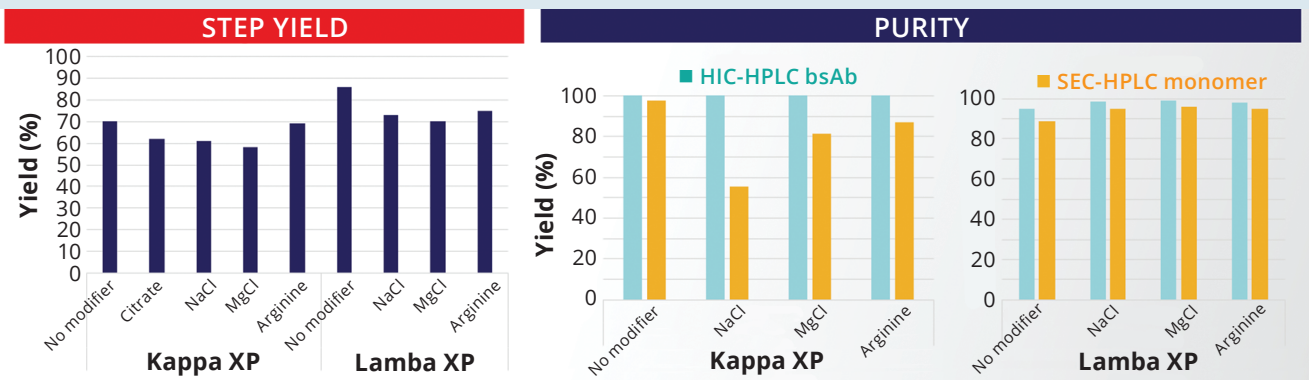


Figure 3: Effect of modifiers on yield and purity of a bispecific antibody (bsAb) using Kappa XP and Lambda XP affinity resins; HIC-HPLC = hydrophobic-interaction high-performance liquid chromatography, SEC-HPLC = size-exclusion high-performance liquid chromatography.



Kappa XP resin and product quality from the Lambda XP resin. Figure 3 summarizes the effects of those modifiers on resin performance.

For the Kappa XP resin, no additional modifier was needed for the bsAb's improved recovery, and purity. There was a significant decrease in purity with the addition of NaCl, which suggests that high salt concentration decreases the stability of the bsAb. For the Lambda XP resin, adding modifiers helped to provide incremental improvements, increasing purity to 98% and reducing aggregation to <5%, which was within an acceptable range. However, recovery decreased from 86% to 70–75%.

Thus, both Kappa XP and Lambda XP resins can be used in a single purification step to purify a bsAb and remove homodimer species in a pH gradient. Based on recovery and purity, the Lambda XP resin emerged as the preferred option, particularly with the addition of modifiers, which improved purity despite a modest reduction in recovery.

Next-Generation Purification

Affinity resins offer high-performance chromatography solutions to help meet purification challenges presented by novel antibody therapeutics and antibody-derivative molecules. CaptureSelect resins enable users to obtain high purities and yields with a single capture step and can be used with mild elution conditions that help preserve product stability. These resins also can reduce the number of process steps required, streamlining workflows and facilitating seamless scale-up to large-scale manufacturing. All these features make CaptureSelect resins an excellent choice for efficient and reliable manufacturing of advanced antibody-based therapeutics.

References

1 Spooner J, et al. Evaluation of Strategies To Control Fab Light Chain Dimer During Mammalian Expression and Purification: A Universal One-Step Process for Purification of Correctly Assembled Fab. *Biotechnol. Bioeng.* 112(7) 2015: 1472–1477; https://doi.org/10.1002/bit.25550.

2 Dong W, Zhang D, Li Y. CaptureSelect FcXP Affinity Medium Exhibits Strong Aggregate Separation Capability. *Prot. Exp. Purif.* 220, August 2024: 106503; https://doi.org/10.1016/j.pep.2024.106503.

3 Rezvani K, et al. Leveraging Light Chain Binding Avidity for Control of Mispaired Byproducts During Production of Asymmetric Bispecific Antibodies. *J. Chromatogr. A* 1683, November 2022: 463533. https://doi.org/10.1016/j.chroma.2022.463533.

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