Practical Guide: Selecting the Optimal Resins for Monoclonal Antibody Process Purification

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Determine Your Optimal mAb Purification Strategy with Our Broad Range of Resins

The development of monoclonal antibody (mAb) technology over the past 25 years has fundamentally changed the questions we ask and led to many innovative discoveries. These highly specific biological products have significantly influenced the direction and progress of research and therapeutics. In research, mAbs are primarily produced and used to isolate, identify, and characterize a specific protein. On the therapeutic front, they have shown promise in treating diseases such as cancer, chronic inflammation, and infection. These applications make mAb purification one of the largest and fastest growing areas of the pharmaceutical industry.

Purification of mAbs relies heavily on column chromatography. However, not all chromatography resins are created equal. Since each resin works within a set range of technical parameters, mAb purification involves multiple consecutive steps using two or more resins. Frequently, these purification steps are referred to as capture, intermediate, and polish. The ideal purification strategy for each mAb is usually customized based on multiple criteria, including its final anticipated use and various purification challenges such as costs, harsh elution conditions, aggregate formation, maximization of monomer recovery, and the desired purity, among other things.

Bio-Rad has provided a progressive selection of chromatography resins for the process-scale purification of biopharmaceuticals for over 50 years. This guide presents the various resins that can be used for process-scale mAb purification and lists the specific features that make them appropriate for those uses.

Capture Purification

UNOsphere SUPrA™ Resin

The first step in mAb purification is their capture from hybridoma cell supernatants. Protein A–based affinity chromatography is by far the most common method for such capture. UNOsphere SUPrA Resin is designed for this process-scale purification (Figure 1). It combines UNOsphere™ bead technology with a recombinant Protein A ligand produced in *E. coli* with animal origin–free reagents. It can be packed at high bed heights for increased residence time (hence improved mAb binding) of the cell extract without excessive pressure spikes. It produces narrow elution profiles, requiring low buffer volumes. Additionally, elutions can be performed at a relatively high pH (≤11), minimizing the potential of aggregate formation/precipitation. This resin provides typically >95% recovery of the target mAb. It also shows relatively good clearing of host cell proteins (HCPs) and DNA. These capabilities, along with its other technical properties (bulletin 5729), make it an ideal candidate for capture purification (bulletin 5728). However, in addition to the relatively high cost of Protein A, one of the limitations of using this as the sole purification resin is some Protein A leaching from the beads that can contaminate the sample.

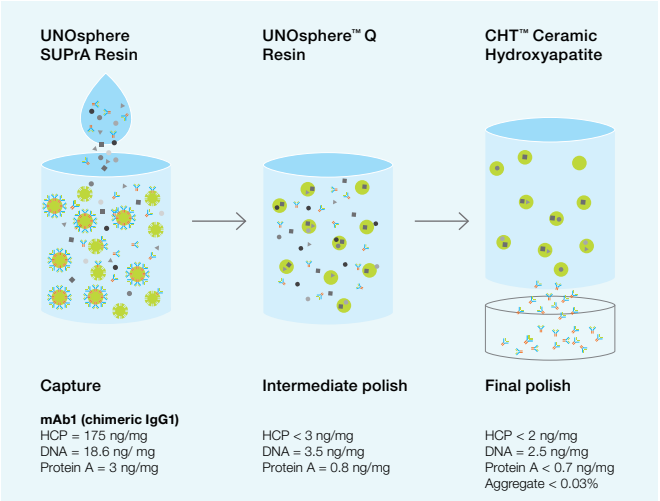

Fig. 1. An example of a mAb purification workflow. Incorporating UNOsphere SUPrA as the capture resin, UNOsphere Q as the intermediate purification resin, and CHT Ceramic Hydroxyapatite as the polish purification media.

Nuvia™ S Resin

This high-capacity strong cation exchanger provides the robustness needed for initial non-affinity–based capture of mAbs (Ng and Snyder 2012; Figure 2) and the resolution needed for an intermediate polishing application. Since it is a Nuvia-based chromatography resin with quaternary sulphonic acid groups attached to polymeric surface extenders, it possesses excellent dynamic binding characteristics over a broad range of pH and conductivity, with low backpressure at the high flow rates required for modern downstream processes. The high flow rates offer the additional advantage of minimizing the antibody's exposure to proteases and nucleases present in the cell culture feedstream. In some cases, the sulphonic acid groups provide better mAb adsorbtion than UNOsphere™ S Resin, which has the same backbone, albeit with no surface-grafted charged polymers (Perez-Almodovar et al. 2012). Its wide conductivity window ensures consistent capture of mAbs, despite the substantial amounts of NaCl required in mammalian expression cultures, and prevents the need for preconditioning or inline dilution before column loading, resulting in smaller column footprints and reduced capital costs. In addition, it is an excellent tool for removing mAb fragments and aggregates (bulletin 5984).

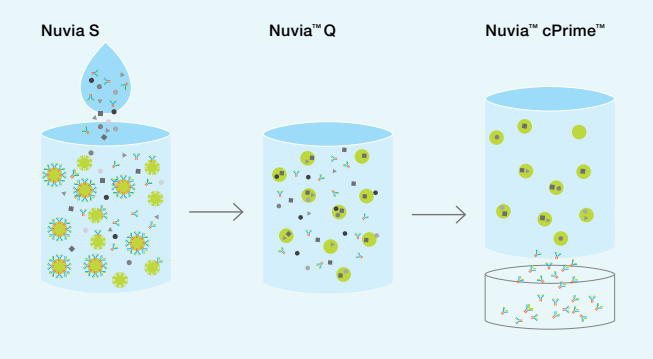

Fig. 2. Initial non-affinity–based capture of mAbs. mAb purification workflow incorporating Nuvia S as the capture resin, Nuvia Q as the intermediate purification resin, and Nuvia cPrime as the polish purification resin.

Intermediate Purification

UNOsphere Q Resin

Ion exchange chromatography is an established step in the purification of mAbs following Protein A affinity capture. UNOsphere Q Resin is a high-capacity, high-throughput anion exchange (AEX) resin that can be used for intermediate purification (Figure 1). This bead is designed with large-diameter pores and high surface area to maximize the capture speed of impurities (HCPs and DNA) from the capture eluant or the initial feed, resulting in high target recovery and productivity. It provides a binding capacity of 125–180 mg protein/ml of resin in the linear velocity range of 150–1,200 cm/hr. Depending on the ultimate purification goal, it can be used in conjunction with a cation exchange (CEX) resin (bulletin 5735) for a more robust purification (Tugcu et al. 2008).

Nuvia Q Resin

This is an ultra high capacity AEX resin for intermediate and polishing steps of mAb purification (Figures 2 and 3) in both affinity-based and affinity-independent workflows. It contains quaternary amino groups attached to proprietary polymeric surface extenders on the Nuvia base matrix, allowing it to have best-in-class dynamic binding capacity over a broad range of pH and flow rates. Most mAbs have an acidic isoelectric point and thus do not bind to AEX resins. Therefore, this AEX resin is ideal for binding to contaminants (bulletin 6745) such as negatively charged HCPs and host cell DNA. A purer fraction of the unbound mAb can be obtained from the flowthrough.

Nuvia™ HR-S Resin

This high-resolution CEX resin can be utilized for both intermediate and final polishing purification steps in different workflows (Figure 3). It is also based on the Nuvia base bead technology and provides the added feature of excellent scalability for large-scale downstream manufacturing. It is ideal for the separation of closely related biomolecules and high molecular weight impurities like mAb aggregates, increasing monomer recovery (bulletin 6439).

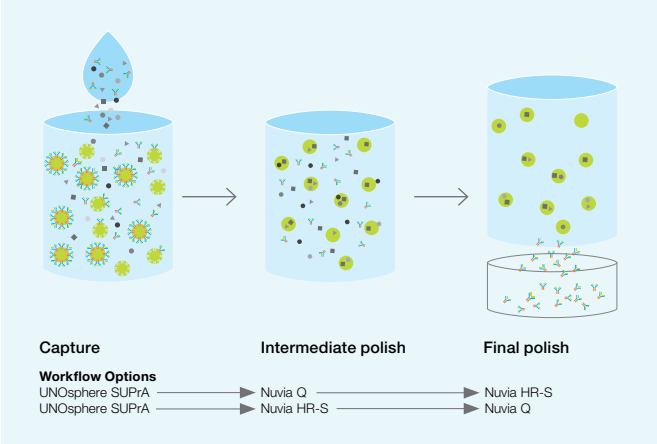

Fig. 3. Flexibility of various resins in mAb purification workflows.

Polish Purification

CHT Ceramic Hydroxyapatite Media

CHT Ceramic Hydroxyapatite is ideal for mAb purification at both intermediate and polish stages (Figure 1). Its unparalleled selectivity enables resolution of mixtures presenting as homogenous by other media. Being a mixed-mode media, CHT provides the dual advantage of CEX and metal affinity purification. It has positively charged calcium binding sites for affinity binding and negatively charged phosphate groups for the CEX interaction. Its diverse selectivity allows for the

highest clearance of aggregates among mixed-mode media and one-step clearance of multiple product-related impurities (bulletin RP0033), including mAb fragments, DNA, HCPs, virus particles, endotoxins, and Protein A. In comparison with other mixed-mode resins, CHT offers the best monomer recovery (bulletin 6749) in the smallest eluate volume (Table 1).

Table 1. CHT offers the best mAb monomer recovery relative to two other comparable resins.

Media	mAb S Monomer Content, %	mAb S Monomer Recovery, %	Eluate Volume, CV
CHT	99.5	82.7	5
Capto adhere	99.5	48.8	14
Capto adhere ImpRes	99.5	61.7	14

Nuvia cPrime Resin

This resin combines hydrophobic and CEX interactions to provide highly robust recovery at high flow rates (Table 2) in commercial manufacturing settings. Its particle size is optimized to deliver exceptional flow properties, fast mass transfer, and stability. It is a salt-tolerant resin and can be effectively used for salt- and pH-sensitive mAb purifications with minimal feed conditioning. Nuvia cPrime has higher affinity for full length mAbs relative to process impurities and by-products and therefore is ideal for the polishing step of mAb purification (Figure 2). It can specifically be used for purification of mAbs that lack an affinity handle. In such a non-affinity–based workflow, it shows maximal clearance of HCPs and host cell dsDNA and minimal aggregate content (bulletin 6241).

Table 2. Nuvia cPrime offers robust mAb recovery at high flow rates.

Flow rate, cm/hr	DBC, 10% BT, mAb X, mg/ml	% Recovery
150	40	88%
200	33	85%
250	30	80%

DBC, dynamic binding capacity; BT, breakthrough.

The workflows and details provided here can help you design a process purification strategy for your mAb. For technical/product support or to request a quote, email your regional Bio-Rad representative at process@bio-rad.com or contact our customer service at 1-800-4-BIORAD (1-800-424-6723).

References

Ng PK and Snyder MA (2012). pH-based cation exchange chromatography in the capture and elution of monoclonal antibodies. J Sep Sci 35, 29–35.

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Explore our extensive selection of process-scale chromatography resins and their performance characteristics and applications (bulletin 6713). For process optimization of your mAb purification, request a sample.

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