Practical Guide: Selecting the Optimal Resins for Immunoglobulin G (IgG) Process Purification

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Comprehensive Solutions for IgG Purification

Immunoglobulins (Ig), or antibodies (Ab), are the heavy globular plasma proteins produced in response to foreign bodies or antigens. They exist in five isoforms: IgA, IgD, IgE, IgG, and IgM. IgG provides the majority of antibody-based immunity. The ability of animal immune systems to produce antibodies against an injected antigen can easily be exploited to generate application-specific antibodies for both basic research and diagnostic applications. Additionally, the proliferation of recombinant DNA and protein technology has opened up previously uncharted territories in antibody construction. Substantial refinements in the upstream expression and production of antibodies have created a need for advanced downstream purification processes to remove contaminants such as albumin, transferrin, and α2-macroglobulin from native serum and host cell proteins (HCPs) from the recombinant source.

A number of factors will affect which chromatography resin you choose to purify the target antibody, including the desired purity for the final application and the required physicochemical characteristics of the purified antibody. Since each resin has unique properties, purification strategies are often broken down into steps such as capture, intermediate, and polish to maximize antibody purity. The resins used at each step have to be selected based on whether they can deliver the desired process-related costs, time lines, and purity, among other factors.

Bio-Rad has provided a progressive selection of chromatography resins for process-scale purification of antibodies for more than 50 years. This guide provides a brief snapshot of the different resins that can be used for the process-scale purification of polyclonal IgGs and the specific features that make them useful for such purifications. For more information about resins appropriate for the purification of mAbs specifically, refer to bulletin 6875 — Selecting the Optimal Resins for Monoclonal Antibody Process Purification.

Affinity Purification

UNOsphere SUPrA™ Resin

Protein A affinity chromatography is widely used for IgG purification since Protein A–based resins bind with high affinity to the Fc regions of most immunoglobulins, including IgG. UNOsphere SUPrA Resin was developed with recombinant Protein A (includes only the IgG binding site to ensure specific pull down of IgG from serum) conjugated to UNOsphere™ beads for robust and scalable IgG purification. This resin provides multiple advantages over other Protein A resins, including predictable performance over a wide range of antibody concentrations and stability at high pH (<11). It has a binding capacity of 25–30 mg/ml of IgG (bulletin 5729) and typically provides >95% recovery of target antibodies at ~90% purity (Figure 1 and Table 1) (bulletin 6053). Therefore, UNOsphere SUPrA is an excellent choice for process-scale purification of IgGs.

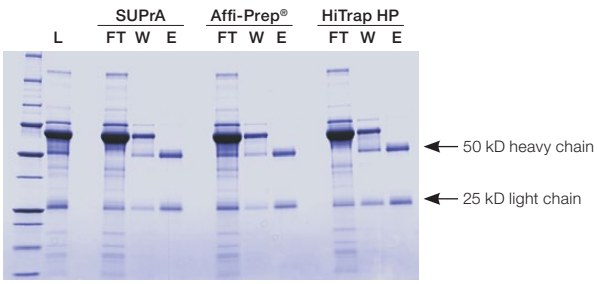

Fig. 1. Purity of IgG obtained with UNOsphere SUPrA Resin relative to two other Protein A resins. L, load; FT, flowthrough; W, wash; E, eluate.

Table 1. Quantitation of remnant HCP and DNA after mAb purification using UNOsphere SUPrA and MabSelect Resins.

Resin	HCP, ng/mg	DNA, ng/mg	rProtein A, ppm
MabSelect	39.2	26.0	6.4
SUPrA	33.2	21.6	20.5

In summary, UNOsphere SUPrA is ideal as a standalone for antibodies that do not have to be exceptionally pure. In addition, it can be used for capture purification followed by other purification steps, which would help in the removal of leached Protein A.

Ion Exchange Chromatography

UNOsphere™ S Resin

UNOsphere S is a cation exchange (CEX) resin that delivers high productivity through high binding capacity and low backpressure. In general, it has been shown to bind 40–60 mg IgG/ml resin in the linear velocity range of 150–600 cm/hr (bulletin 2669). More specifically, it has been used successfully in the capture purification of a recombinant antibody from corn seeds with 97% recovery and 80% purity (bulletin 2774) (Table 2) and the high efficiency capture of murine IgG_1 with a demonstrated recovery of 97% (Figure 2). It possesses a unique ability to self-generate a pH gradient during certain elution schemes, which gives it a resolving power unattainable by other CEX resins. Additionally, harsh clean-in-place and sanitization cycles do not affect its long-term stability, making it very amenable to regeneration and reuse. This resin has also been shown to successfully clear Protein A contamination (bulletin 2849).

Table 2. Summary of transgenic IgG purification by UNOsphere S and CHT™ Ceramic Hydroxyapatite.

Purification Step	Total IgG, mg	Total Protein, mg	Recovery, %	Purity, %
Crude corn extract + IgG	1.0	6.1	100	22
UNOsphere S	1.07	1.47	97	80
CHT	0.88	0.91	82	95

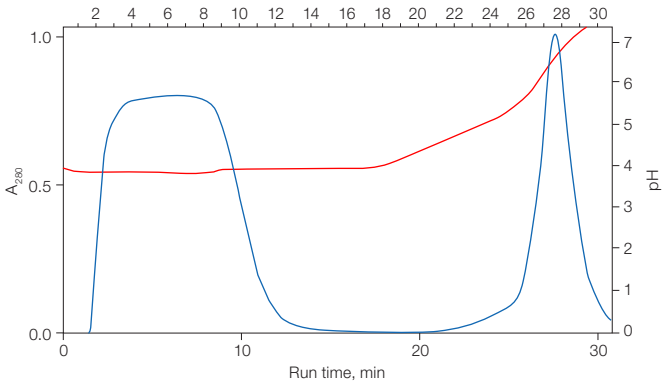

Fig. 2. Purification of murine IgG_1 on a UNOsphere S Column. A_{280} (—); buffer pH (—).

Macro-Prep® DEAE Resin

Macro-Prep DEAE is a weak anion exchange (AEX) resin with excellent flow properties and the ability to maintain its resolving power at flow rates ≥1,000 cm/hr. Most IgGs have a basic isoelectric point and thus do not bind to AEX resins. Therefore, this AEX resin serves well in binding contaminants such as negatively charged HCPs and host cell DNA, ensuring that a purer fraction of unbound basic IgG can be obtained from the flowthrough. Macro-Prep DEAE Resin is stable in a wide variety of organic solvents, making it possible to thoroughly sanitize and regenerate the material. It operates well at low and medium pressures and exhibits minimal volume changes in response to variations in pH or ionic strength. It has been used as a capture purification resin for horse IgG_T (bulletin 2524).

Mixed-Mode Chromatography

CHT Ceramic Hydroxyapatite Media

CHT offers unique selectivities, enabling the resolution of mixtures that appear homogenous with other media. It is an excellent choice for intermediate or final polishing. Its robust properties improve IgG purification quality, efficiency, yield, and financial value through its large capacity for high-titer upstream feedstocks. CHT Media is available in two formats, Type I and Type II. In addition to increasing purity, CHT has also been shown to decrease the amount of IgG_4 aggregates from a biopharmaceutical product (Figure 3) (bulletin 2940). Furthermore, CHT can also be utilized for the removal of Protein A contaminants (bulletin 2849), which is advantageous due to the wide range of immunomodulatory effects associated with Protein A and its fragments. It provides easy process-scale separation of Protein A from unfractionated conditioned media without the need for additional steps or agents. CHT can also be used for endotoxin removal from feed using a 0–1 M phosphate gradient (bulletin RP0033).

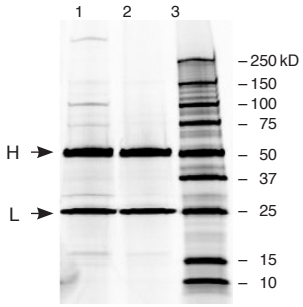

Fig. 3. Reducing SDS-PAGE analysis of samples after CHT Column purification. Lane 1, IgG_4 starting material; lane 2, CHT-purified sample; lane 3, MW standards; H, IgG_4 heavy chain; L, IgG_4 light chain.

CFT™ Ceramic Fluoroapatite Media

CFT is an apatite-based media that is a composite of fluoroapatite and hydroxyapatite. One of its unique properties is that it can be used in low pH (~5.0) purifications. Its tensile strength, chemical durability, and density provide excellent throughput and consistent performance for IgG separations. It can be used for the polish step in IgG purification after the target molecules have been captured and eluted from a Protein A column (bulletin 5853).

For the purification of monoclonal IgG, other resins such as Nuvia™ Q, Nuvia™ S, Nuvia™ HR-S, and Nuvia™ cPrime™ can be used as well. See bulletin 6875 for details about these resins in the purification of monoclonal IgG.

The details provided here can help you design a process purification strategy for your polyclonal IgG. 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).

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

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