Practical Guide: Selecting the Optimal Resins for the Process Purification of Native and Recombinant Proteins

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Accelerate Your Protein Purification Workflow with Our Broad Range of Process Resins

Native and recombinant proteins are used in a variety of applications ranging from drug discovery to target validation to high-throughput screening. Due to this, large-scale protein production and purification have become major necessities in industrial settings. Since the approval of recombinant insulin as a therapeutic in the early 1980s, the number of protein-based biotherapeutics has been on a constant rise. Currently, more than 200 proteins have been approved for human therapeutic and diagnostic use and over 350 are in late-stage clinical trials (Walsh 2010). In addition, large-scale production strategies yield cell culture titers containing tens of grams of target protein per liter. The escalating demands for higher protein titers have therefore been successfully met. However, downstream processes must still ensure that impurities are removed. These processes, including purification, represent 45–92% of the total cost of manufacturing a pure batch of recombinant protein (Straathof 2011). Therefore, devising efficient and economical purification strategies is an absolute requirement.

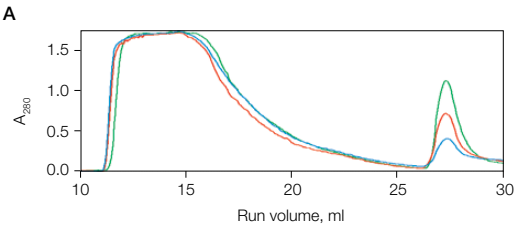
Bio-Rad has provided a progressive selection of chromatography resins for process-scale purification of proteins for over 50 years. This guide highlights these resins and their chromatographic types and lists the specific features that make them appropriate for use in process protein purification.

Affinity Chromatography

Immobilized metal affinity chromatography (IMAC) is commonly used for histidine-tagged recombinant protein purification. Several therapeutic candidate proteins purified using IMAC are currently in clinical trials. IMAC uses a matrix that has a metal-chelating group available for metal binding. The immobilized metal contains coordination sites that can bind to histidine residues attached to the recombinant protein, thus enabling its purification via elution with a metal chelator such as imidazole.

Profinity™ IMAC Resins

Profinity IMAC Resins are based on our patented UNOsphere™ bead technology. They have excellent pressure flow properties for high maximum operating pressures and high flow rates, which allow rapid purification, column cleaning, and re-equilibration. They exhibit superb mechanical strength and are compatible with denaturing agents, detergents, and reducing agents. Their yield and purity is superior to IMAC resins from other commercial suppliers (Figure 1) (bulletin 5456). Additionally, they are available in two forms — uncharged and precharged with Ni^{2+}. The uncharged form can be charged with any metal ion for even greater purification flexibility.



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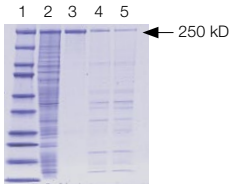

C

Lane	Resin	Yield	Purity
3	Profinity IMAC	3.6 mg	86%
4	Supplier A	2.1 mg	36%
5	Supplier B	1.1 mg	20%

Fig. 1. Comparison of Bio-Rad's IMAC resin to IMAC resins from two other commercial suppliers. A, overlaid chromatograms. **B**, SDS-PAGE analysis of the histidine-tagged proteins. **C**, calculated yield and purity of the eluted histidine-tagged proteins. Profinity IMAC (–); supplier A, (–); supplier B (–).

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Ion Exchange Chromatography (IEX)

IEX is one of the most frequently implemented initial steps for protein purification, provided that the target protein is untagged. Proteins are amphoteric molecules and can as such bind to an IEX resin, depending on the pH of the solution. The binding or elution buffer pH does not affect the charge on strong ion exchangers, which retain their charge over a wide pH range. Hence, they are usually the resins of choice in commercial bioprocess operations. Elution of proteins can be performed by altering the pH or ionic strength of the solution. However, when altering the pH, there can be less control on the reproducibility. Therefore, for large-scale studies, a salt gradient is preferred. Theoretically, any salt can be used for elution since they all modulate electrostatic interactions and thus binding and elution. However, salting out can induce proteins to bind to resins more strongly, so salts such as sodium chloride (NaCl), which have a weak salting-out effect, are selected preferentially over high salting-out salts such as ammonium sulfate $(NH_4)_2SO_4$.

Bio-Rad offers numerous IEX resins that have been shown to be very efficient in process protein purification.

Nuvia™ Ion Exchange Resins

Nuvia IEX Resins are designed to provide high binding capacities, exceptional resolution, and high productivity. To highlight their protein purification capabilities, we performed rapid single-step protein purification from whey. The major whey proteins include α-lactalbumin (ALA), β-lactoglobulin (BLG), bovine serum albumin (BSA), and bovine immunoglobulins. These were purified using a workflow with Nuvia™ S and Nuvia™ Q Resins (Figure 2) (bulletin 6128).

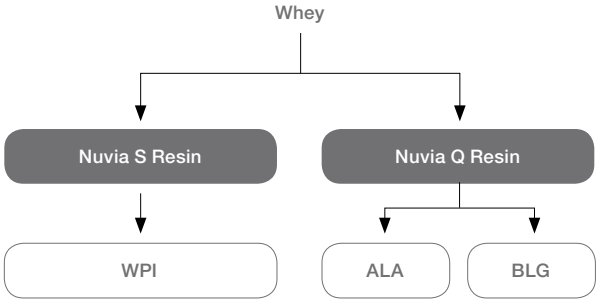


Fig. 2. Workflow for protein purification from whey using Nuvia S and Nuvia Q IEX Resins. WPI, whey protein isolate.

Nuvia Q Anion Exchange (AEX) Resins

These are an ultra–high capacity resin with high binding capacity over a range of pH and flow rates. They were used to efficiently purify ALA and BLG from whey with a densitometric purity of 85%. They have also been shown to be useful in purifying proteins such as transthyretin (TTR) (bulletin 6711). Recombinant TTR purification is problematic due to its aggregation properties, which prevent the use of acidic buffers or denaturation agents. Since TTR has an isoelectric point of 5.7, Nuvia Q could be used to purify it under neutral buffer conditions. The resultant fractions were >95% pure (Figure 3).

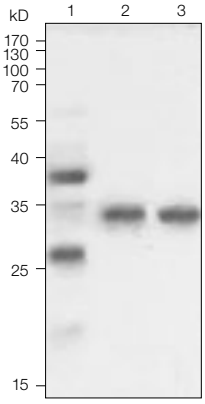


Fig. 3. SDS-PAGE analysis of TTR. Lane 1, protein contaminants; lane 2, eluted TTR; lane 3, TTR standard.

Nuvia S Cation Exchange (CEX) Resins

These are ultra high capacity resins that provide very high capture capacities and exceptional flow properties designed to meet process purification needs. They have been shown to efficiently purify whey protein isolate (WPI) from whey. Since most whey proteins have an isoelectric point of 4.7, loading whey onto Nuvia S Resin at pH 4.0 resulted in capture of WPI, with all the other proteins in the flowthrough. WPI was eluted with an elution buffer at pH 8.0.

UNOsphere IEX Resins

These resins are versatile in both capacity and resolution and provide a cost effective solution to protein purification. They are designed for high-efficiency capture of biomolecules from crude feedstreams. UNOsphere™ Q AEX Resins have a large surface area to maximize capture speed, macromolecule capacity, recovery, and productivity. They exhibit binding capacities as high as 125–180 mg BSA/ml resin at a linear velocity range of 150–1,200 cm/hr (bulletin 2724). UNOsphere™ S CEX Resins exhibit high batch-to-batch reproducibility and possess a unique ability to self-generate a pH gradient during certain elution schemes (bulletin 2669).

Macro-Prep® IEX Resins

These resins have unique performance capabilities for large biomolecule purification, including plasma proteins and virus-like particles, due to the large pore structure and mixed-mode character of a slightly hydrophobic polymer backbone with hydrophilic ligands. This family contains four different types of IEX resins for process purification. Macro-Prep® High S Resins are strong CEX resins ideal for purifying basic and neutral proteins and peptides and can meet rapid purification challenges (bulletin 5643). Macro-Prep® CM Resins are weak CEX resins that provide consistent and reproducible results (bulletin LIT271). Macro-Prep® High Q Resins are strong AEX resins ideal for purifying acidic and neutral proteins and peptides (bulletin 2204). Macro-Prep® DEAE Resins are weak AEX

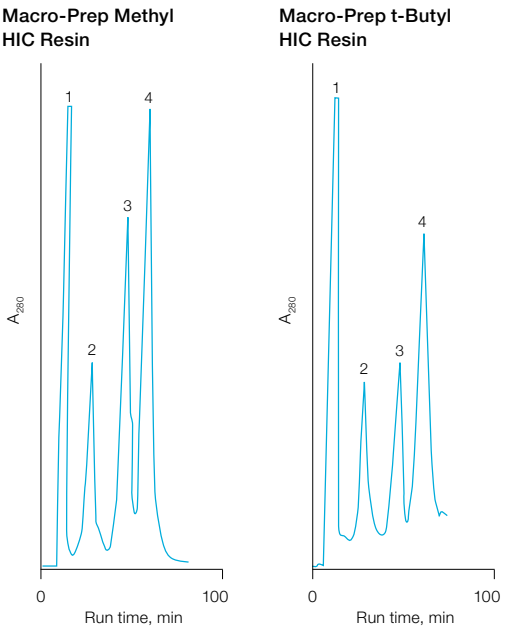
resins that have high chemical, mechanical, and thermal stability and meet the demands of analytical, semipreparative, and process-scale applications (bulletin 1942).

Hydrophobic Interaction Chromatography (HIC) Resins

Protein hydrophobicity is determined by its amino acid constitution. High salt concentrations expose surface hydrophobic patches, enabling proteins to bind to the alkyl group of HIC resins. The proteins can then be eluted out by decreasing the salt gradient. HIC is often used in conjunction with IEX in protein purification workflows. Since IEX resins elute in high salt conditions and HIC binds proteins under such conditions, HIC can be used following an IEX step without the requirement of a buffer exchange.

Macro-Prep® HIC Resins

We offer methacrylate-based Macro-Prep HIC Beads for protein purification using hydrophobic interactions. Both methyl and t-butyl resins are available for purifying proteins that have weak or strong hydrophobic regions. The retention, selectivity, and biological activity of the proteins are dependent on the pH and type of salt used and its concentration. The two different ligands provide alternative selectivities for easier optimization of separation (Figure 4) (bulletin 1841).



		Retention Times, min	
Peak	Protein	Methyl	t-Butyl
1	Cytochrome c	7.30	10.41
2	Ovalbumin	32.25	40.22
3	α-Amylase	62.81	71.50
4	Ferritin	81.49	90.90

Fig. 4. Comparison of the separation of proteins and their retention times.

Mixed-Mode Chromatography Resins

Mixed-mode resins/media offer unique selectivities. These resins combine the properties of CEX reins and metal affinity or HIC resins, often enabling the separation of biomolecules that appear homogeneous using other chromatographic methods. Such features enhance the popularity of mixed-mode resins in protein purification schemes. We offer two types of mixed-mode resins/media — CHT™ Ceramic Hydroxyapatite and Nuvia™ cPrime™.

CHT Ceramic Hydroxyapatite Media

CHT has some robust properties that improve efficiency, yield, and financial value. Two types of CHT Ceramic Hydroxyapatite are available, Type I and Type II. Type I has a higher protein binding capacity and better capacity for acidic proteins. Type II has a lower protein binding capacity but offers better resolution of nucleic acids and certain proteins. It is possible to manipulate the binding and retention of different proteins by changing the buffer pH and the type of CHT used, as we have shown with 16 different proteins (Figure 5) (bulletin 1986).

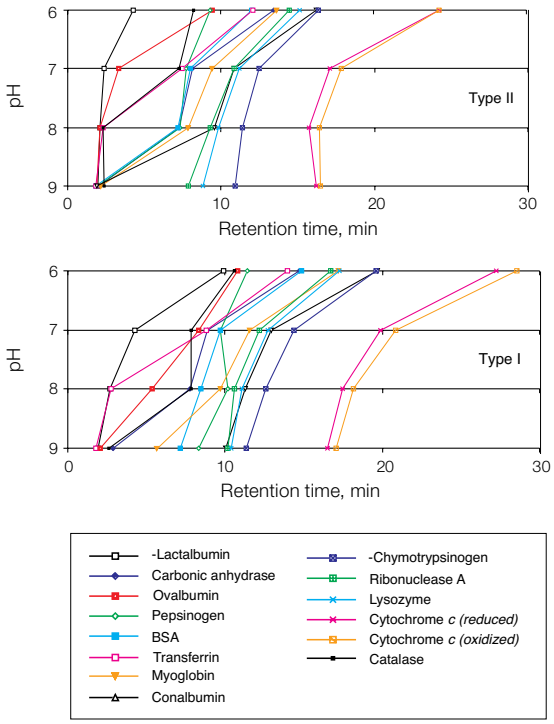


Fig. 5. Changes in retention time on CHT Type I and Type II Media with increasing pH. The purified proteins were loaded and eluted using a linear gradient of sodium phosphate at pH 6, 7, 8, and 9.

CHT complements other forms of chromatography very efficiently to form a complete workflow for protein purification. For example, we have shown that CHT purification following an IMAC step generates highly purified alpha 1-antitrypsin samples (bulletin 2535). Furthermore, it can also be used for single-step purification of native proteins, which are untagged and hence cannot be purified via IMAC. We have also used CHT in a single-step purification to isolate frequenin, a calcium binding protein (bulletin 2575). Many other proteins have been shown to be purified by CHT in a single step, including calretinin, calbindin, and a calcium-dependent adhesin from Rhizobiaceae adhesion (Cheung et al. 1993, Smit et al. 1989).

Nuvia cPrime Resins

These resins were specifically designed for process-scale purification of a wide variety of therapeutic proteins and diverse new constructs, many of which lack an affinity handle. They allow hydrophobic and CEX interaction modes to achieve effective purification. These resins have a large design space for binding and elution, allowing for the development of highly robust methods in a commercial manufacturing setting. Salt- and pH-sensitive proteins with a high propensity for aggregation and/or degradation can be effectively purified using Nuvia cPrime. A diverse set of proteins with different isoelectric points (pI) have been shown to be purified on these resins using different conditions (Table 1) (bulletin 6418).

Table 1. Optimal binding and elution conditions of various proteins on Nuvia cPrime.

Test Protein	pI	Optimal Conditions Predicted by DoE
Bovine serum albumin	4.7	Binding: 10 mM NaCl, pH 4.0 Elution: 1,000 mM NaCl, pH 8.0
Bovine carbonic anhydrase	5.9	Binding: 10 mM NaCl, pH 4.6 Elution: 1,000 mM NaCl, pH 8.0
Conalbumin	6.9	Binding: 10 mM NaCl, pH 4.0 Elution: 505 mM NaCl, pH 6.0
Lactoferrin	9.2	Binding: 205 mM NaCl, pH 4.0 Elution: 1,000 mM NaCl, pH 8.0
mAb X	9.5	Binding: 300 mM NaCl, pH 4.6 Elution: 800 mM NaCl, pH 8.0

The details provided here can help you design a process purification strategy for your recombinant proteins. 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

Cheung W-T et al. (1993). Calcium binding by chick calretinin and rat calbindin D28k synthesised in bacteria. Eur J Biochem 215, 401–410.

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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 native and recombinant protein purification, request a sample.

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