Process Resin Selection Guide

BIO-RAD

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BIO-RAD

INTRODUCTION

History of Chromatography Resins at Bio-Rad

Bio-Rad has provided chromatography resins for lab-scale analysis and process-scale purification of biopharmaceuticals for over 50 years.

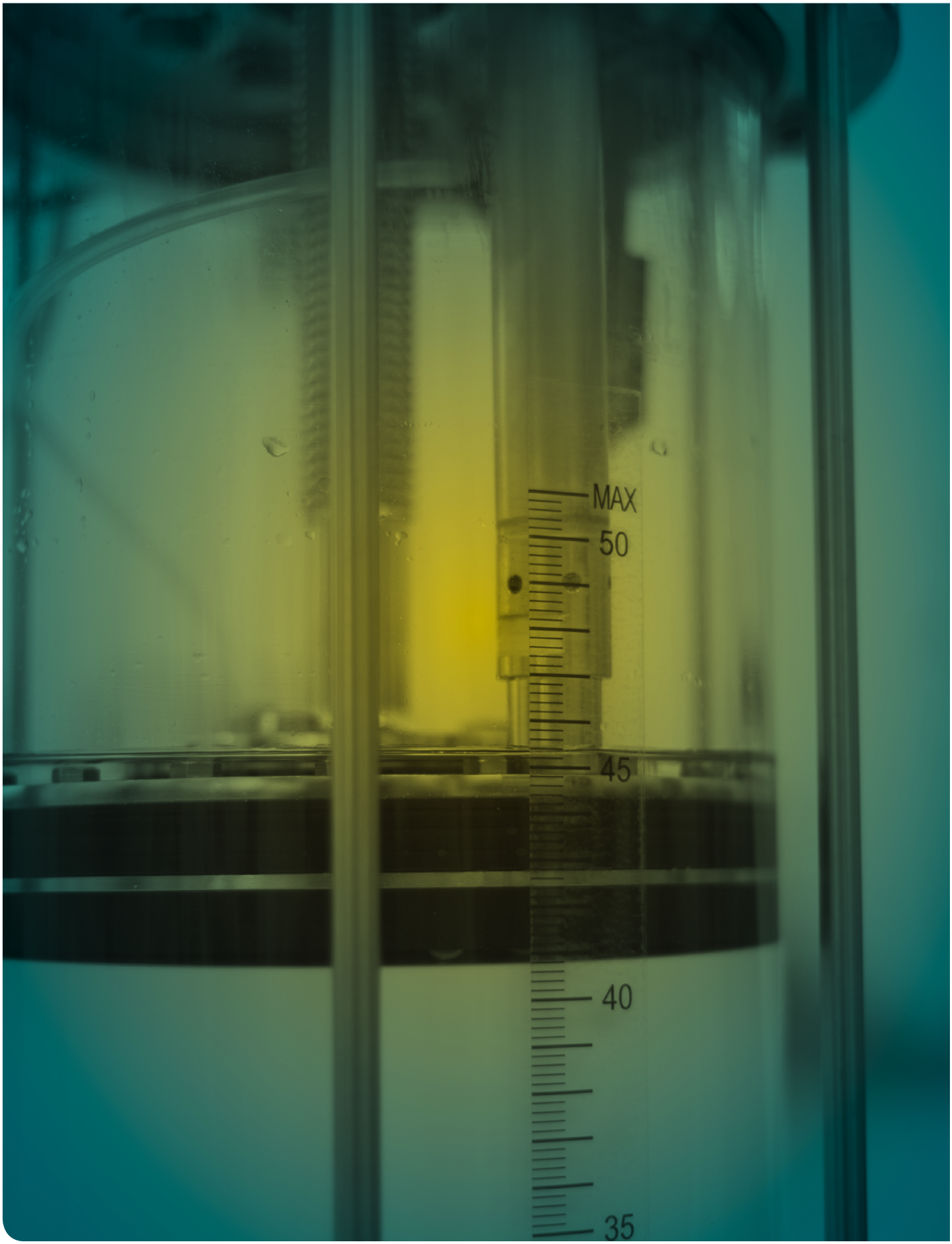
Bio-Rad's initial polymer-based resins included polystyrene ion exchange, size exclusion, and hydrophobic adsorption resins. These are used for the removal of trace impurities from therapeutic proteins, pre-use purification of bioprocessing buffers, and purification of compounds less than 3,000 daltons. During the 1960s and 1970s, Bio-Rad developed polyacrylamide- and agarose-based chromatography resins, which have been used for size exclusion, immunoprecipitation, and affinity chromatography.

The company introduced crystalline hydroxyapatite in 1956 for protein purification and nucleic acid separations. This expertise enabled Bio-Rad to become the exclusive worldwide supplier of CHT™ Ceramic Hydroxyapatite in the early 1990s. CHT exhibits unique selectivity for a wide variety of biomolecules, which allows it to achieve effective separation of antibodies from antibody aggregates, host cell proteins, DNA, and endotoxins.

During the 1980s, Bio-Rad introduced 25 and 50 µm rigid methacrylate-based Macro-Prep® Ion Exchange, Macro-Prep Hydrophobic Interaction Chromatography, and affinity resins, which provide high flow rate separations at low and medium pressures. The combination of large pores, good flow properties, and a mild hydrophobic effect makes Macro-Prep Ion Exchange Resins particularly useful for plasma protein fractionation.

Since 2000, to meet the needs of the biopharmaceutical industry for high binding capacity, fast capture, and high resolution, Bio-Rad has developed UNOsphere™ and Nuvia™ Ion Exchange, Affinity, and Mixed-Mode Resins. These resins are based on a hydrophilic polymer produced from water-soluble acrylamido and vinylic monomers, leading to low nonspecific binding due to the hydrophilic nature of the monomers and polymer. UNOsphere and Nuvia Resins are available as either high-capacity or high-resolution ion exchange resins. Nuvia™ cPrime™ Resin, a hydrophobic cation exchange mixed-mode resin, provides unique selectivity for proteins and is easily combined with CHT for excellent purification of various types of biomolecules.

The broad range of resins, combined with Bio-Rad's long-standing commitment to customer support and technical depth, provide researchers and biopharmaceutical manufacturers with a partner unequaled in the industry.


MAX
50
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40
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CHAPTER 1

Resin Families

Base Bead Overview

Base Bead Chemistry	Product	Particle Size, µm	Derivatives	Scale
Ceramic Apatite	CHT™	20, 40, 80	Hydroxyapatite, Type I and Type II	Lab to bioproduction scale (40, 80 µm); Analytical to lab scale (20 µm)
	MPC™	40	Partially fluorinated, Type I	Lab to bioproduction scale
	CFT™	40	Fluorinated, Type II	Lab to bioproduction scale
Crystalline Apatite	Bio-Gel® HT (P)	40–80		Analytical to lab scale
Acrylamido polymer	Nuvia	50, 70, 85	IEX, mixed-mode, IMAC	Lab to bioproduction scale
	UNOsphere	80, 120	IEX, Protein A	Lab to bioproduction scale
	UNO®	Monolith	IEX	Analytical scale
	Bio-Gel P	40–80	SEC	Analytical to lab scale
Polymethacrylate	Macro-Prep	25, 50	IEX, HIC, affinity	Lab to bioproduction scale
	Affi-Prep®	50	Affinity, Protein A	Analytical to lab scale
	ENrich™	10, 11	IEX, SEC	Analytical to lab scale
Agarose	Bio-Gel A	40–80	SEC	Analytical to lab scale
	Affi-Gel®	50–300	Affinity, Protein A	Analytical to lab scale
Polystyrene	AG®	40–1,200	IEX, mixed-mode	Analytical to bioproduction scale
	Bio-Rex™	40–1,200	IEX	Analytical to bioproduction scale
	Chelex®	40–1,200	Chelating resin	Analytical to bioproduction scale
	Aminex®	9, 25, 30	IEX, ion exclusion, size exclusion, normal/reverse phase, ligand exchange derivatives for Aminex	Analytical scale
	Bio-Beads™	40–80	HIC, SEC	Analytical to bioproduction scale

Abbreviations

AEX – anion exchange
AU – absorbance units
BSA – bovine serum albumin
BT – breakthrough
CEX – cation exchange
CIP – clean-in-place
CV – column volume
DBC – dynamic binding capacity
DI – deionized
DoE – design of experiments
GLC-MS – gas-liquid chromatography MS
HIC – hydrophobic interaction chromatography
hIgG – human immunoglobulin G
HPLC – high-performance liquid chromatography
HSA – human serum albumin
ID – inner diameter
IEX – ion exchange
IgG – immunoglobulin G
IMAC – immobilized metal affinity chromatography
mAb – monoclonal antibody
MM – mixed mode
MS – mass spectrometry
PAH – polycyclic aromatic hydrocarbon
PBS – phosphate buffered saline
PCB – polychlorinated biphenyls
PEG – polyethylene glycol
pI – isolelectric point
PVC – polyvinyl chloride
SDVB – styrene/divinylbenzene
SEC – size exclusion chromatography

Bio-Rad Process Separations

For technical/application support, sales, or inquiries, please contact us at:

Email: process@bio-rad.com
Customer Service: 1-800-4-BIORAD (1-800-424-6723)
Technical Support: 1-800-4-BIORAD (1-800-424-6723)

Visit **bio-rad.com/resin-library** to request a sample and for additional resources about Bio-Rad Process Chromatography Solutions.

INTRODUCTION

Bio-Rad offers numerous chromatography products for the purification and characterization of biomolecules such as proteins, peptides, and nucleic acids. A resin manufacturer for over 50 years, Bio-Rad has expertise in investigating and manipulating pore structure, bead rigidity, mass transfer rate, ligand structures, and ligand density. These parameters are adjusted so that our chromatography beads provide optimal performance in the purification of biopharmaceuticals. Such characteristics are modulated for the production of both analytical grade and process-scale resins. Process-scale resins provide excellent flow rates when packed in columns of diameters up to 2 m and stability over many CIP cycles. The selectivity provided by the process-scale resins is also useful for laboratory separations with the best resolution often provided by the smaller bead/analytical grade resin.

With the innovation of a spherical ceramic form of crystalline hydroxyapatite, CHT Ceramic Hydroxyapatite, Bio-Rad brought this high-performance material to the biopharmaceutical and diagnostics production industries. Similarly, Bio-Rad introduced monolith technology for high-resolution, high-flow laboratory-scale purifications in 1997. This revolutionary material is polymerized along with the functional group in a single step of a highly controlled process. The UNO monolith, with its rapid mass transfer, large interconnected flow channels, and consistent manufacturing process, was ideal at small scale but could not readily be manufactured for large-scale bioprocessing applications for commercial-scale use. Therefore, Bio-Rad converted the acrylamido polymer chemistry into a bead form and introduced the UNOsphere ion exchangers. By taking advantage of the single-step polymerization chemistry for the monolith, Bio-Rad was able to make UNOsphere ion exchange beads with reproducible and consistent properties. Using other monomers during polymerization, Bio-Rad found that activated forms of the base bead (Nuvia) could be manufactured to allow coupling of any desired ligand, greatly expanding the choices that process developers could leverage.

Nuvia™ Resins

High-performance premium products meeting distinct purification challenges

Composition and Properties

Polymer	Properties	Derivatives
▪ Macroporous hydrophilic acrylamido polymer derivatives ▪ Some chemistries include surface extenders, which increase binding capacity ▪ Low carboxyl content in the polymer backbone	▪ Average particle size range from 50 to 85 µm ▪ Pore structure can be gel phase or open pore depending on chemistry ▪ Constant DBC over a wide range of flow rates (up to 500 cm/hr) ▪ Low nonspecific binding due to hydrophilic nature of polymer	▪ High capacity S and Q (includes surface extenders) ▪ Mixed mode ▪ High-resolution S ▪ IMAC

Advantages

Chromatographer	Engineer	Economic buyer
▪ Diverse functionality to meet purification needs all based on high-performance base matrix ▪ Designed to have few to no nonspecific interactions, resulting in predictable performance ▪ Fast mass transfer allows low residence time without loss of DBC	▪ Fast mass transfer ▪ Small elution volumes (3–6 CV) ▪ Chemical and mechanical stability ▪ Short cycle times ▪ Exceptional lot-to-lot reproducibility ▪ Easy to pack	▪ Withstands multiple CIP cycles ▪ Robust chemical stability ▪ High capacity for smaller column footprint

INTRODUCTION

Nuvia Resins are designed to meet specific purification challenges. For example, Nuvia S provides high binding capacity with salt tolerance and is recommended for nonaffinity capture. Nuvia HR-S provides high-resolution CEX polishing for removal of closely related impurities. Nuvia™ cPrime™ provides unique selectivity with both cation and hydrophobic interactions. Nuvia-based beads are produced using controlled polymerization of water-soluble acrylamido and vinylic monomers and exhibit low nonspecific binding due to the hydrophilic nature of the polymers. The average particle size of 50–85 µm provides excellent pressure-flow properties with protein binding capacity maintained at flow rates up to 500 cm/hr. Nuvia Resins exhibit excellent stability with extended exposure to 1 N NaOH.

CATION EXCHANGE

NUVIA RESINS

Nuvia™ S Resin

FUNCTIONALITY
- High-capacity capture
- High salt tolerance
- Non–affinity-based platform purifications

Nuvia S is an ultra high capacity, innovative CEX resin built on the industry proven Nuvia base matrix technology. Nuvia S contains quaternary sulfonic acid groups attached to polymeric surface extenders. The resin delivers superior binding capacity over a broad range of pH, conductivity, and flow rates, providing flexible process design for both capture and polishing of therapeutic proteins. Nuvia S delivers value and flexibility by allowing users to achieve high binding capacity at fast flow rates and by using less resin and smaller column footprints. Nuvia S can significantly improve productivity by contributing to reduced capital costs, buffer consumption, space requirements, and cycle time for downstream processes.

Benefits

- Ultra-high binding capacity for biomolecules at high flow rates
- High binding performance over a wide operating window
- High performance for nonaffinity capture

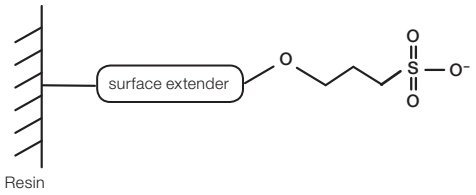


Fig. 1. Nuvia S Resin.

Bead Properties

Property	Description
Type of ion exchanger	Strong cation
Functional group	$-SO_3^-$
Particle size	85 ± 15 µm
Total ionic capacity	90–150 µeq/ml
Dynamic binding capacity	>110 mg/ml at 300 cm/hr 10% BT capacity determined with 4.5 mg/ml hIgG in 40 mM Na acetate + 30 mM NaCl, pH 5.0.
Recommended linear flow rate	50–300 cm/hr
Pressure vs. flow performance	Under 2.5 bar at a flow rate of 600 cm/hr in DI water (20 x 20 cm packed bed, 1.17 compression factor)
Compression factor (settled bed volume/ packed bed volume)	1.15–1.20
pH stability	Short term: 2–14 Long term: 4–13
Shipping solution	20% ethanol + 0.1 M NaCl
Regeneration	1–2 M NaCl
Sanitization	0.5–1.0 N NaOH
Storage conditions	20% ethanol or 0.1 N NaOH
Chemical stability	
1.0 N NaOH (20°C)	Up to 1 week
0.1 N NaOH (20°C)	Up to 5 years
Shelf life	5 years

Binding Capacity

Nuvia S is designed to meet the pressure-flow requirements of demanding purification processes. With optimized bead size distribution and mechanical properties, Nuvia S consistently delivers very high binding capacity at fast linear velocities while maintaining low column pressure (Figure 2). The wide operating window allows users to operate at increasingly higher flow rates without compromising binding capacity.

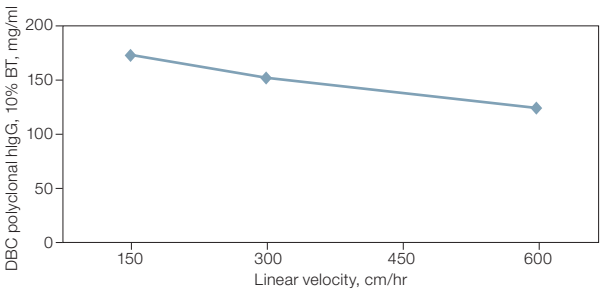


Fig. 2. Dynamic binding capacity vs. flow velocity. A 1.1 cm diameter column was packed to a 20 cm bed height with Nuvia S Resin. Polyclonal hIgG (4.5 mg/ml) in 40 mM NaOAc and 30 mM NaCl, pH 5.0, was loaded until 10% BT was observed.

Nuvia S is designed with proprietary surface extender technology and tuned for optimized charge, flow, and binding kinetics. The unique resin design gives Nuvia S best-in-class dynamic binding characteristics (Figure 3).

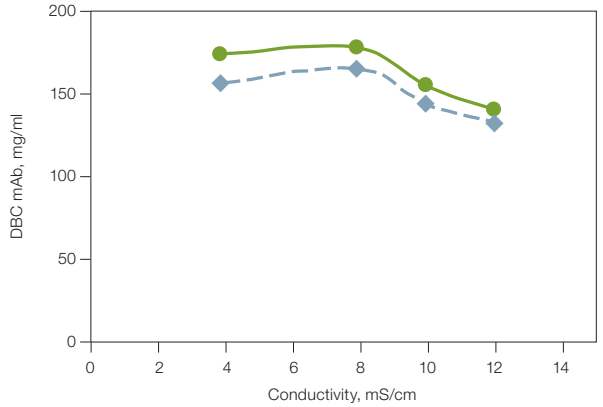


Fig. 3. Binding of a mAb by Nuvia S Resin. Column size, 1.1 x 10 cm. The sample mAb (4.5–5.0 mg/ml) was loaded onto the column in 20 mM NaOAc, pH 5.0 + NaCl (adjusted for conductivity) until 5% (◆) and 10% (●) BT capacity were observed.

Pressure/Flow Performance

Figure 4 shows the effect of flow rate and load viscosity on Nuvia S Resin backpressure.

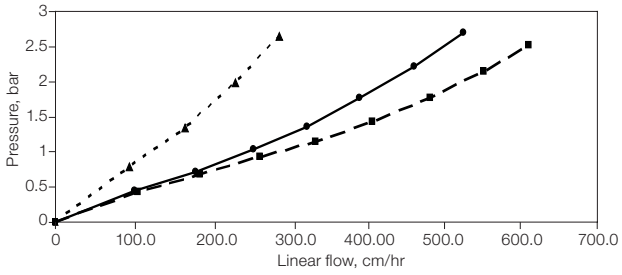


Fig. 4. Nuvia S Resin pressure/flow performance. A 20 cm diameter x 20 cm bed height column was packed to a compression factor of 1.17. Water (–◆–); 10% glycerol (—●—); 30% glycerol (- -▲- -).

Performance Data

High Productivity

The binding capacity of polyclonal hIgG to Nuvia S was compared to that of other commercially available CEX resins (Figure 5).

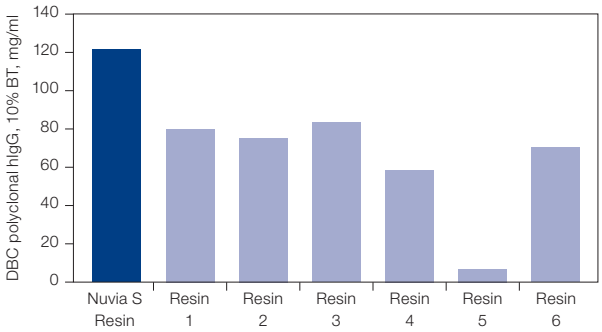


Fig. 5. Binding of polyclonal hIgG by Nuvia S Resin. Comparison of Nuvia S and other commercially available CEX resins. Column size, 0.7 x 5.5 cm. The sample was loaded onto the column in 40 mM NaOAc, pH 5.0 + 30 mM NaCl, washed, and then eluted with 40 mM NaOAc, pH 5.0 + 1 M NaCl.

Nuvia S, like all Nuvia- and UNOsphere™-based chromatography resins, is characterized by its excellent flow properties and fast mass transfer. Surface extenders grafted into the pore space of the Nuvia S matrix make the sulfonic acid ligands more available to electrostatic interaction with opposite charges on the incoming antibody molecules. As shown in Figure 2, Nuvia S exhibits efficient binding for hIgG in the linear velocity range of 150–600 cm/hr, significantly outperforming all commercial CEX resins tested (Figure 6). The hIgG binding capacity on Nuvia S was found to be 114 mg/ml even at a high flow rate of 600 cm/hr. Such performance allows Nuvia S to deliver the speed and throughput needed in process manufacturing of mAbs. For initial capture, operation at high flow rates offers the additional advantage of minimizing the target antibody's exposure to proteases and nucleases present in the cell culture feedstream.

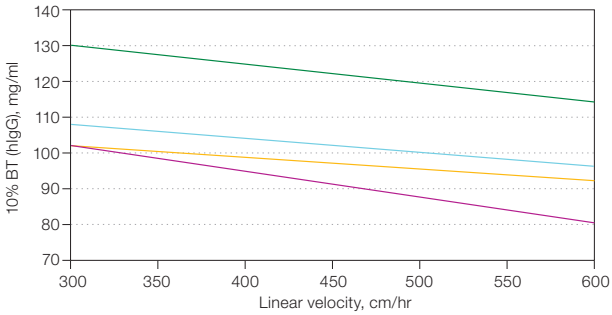


Fig. 6. Binding of hIgG by Nuvia S Resin at various linear velocities. Comparison of Nuvia S with commercially available CEX resins. Nuvia S (—); CEX 1 (—); CEX 2 (—); CEX 3 (—). Column size, 1.1 x 9.5 ± 0.3 cm.

Nonaffinity Capture

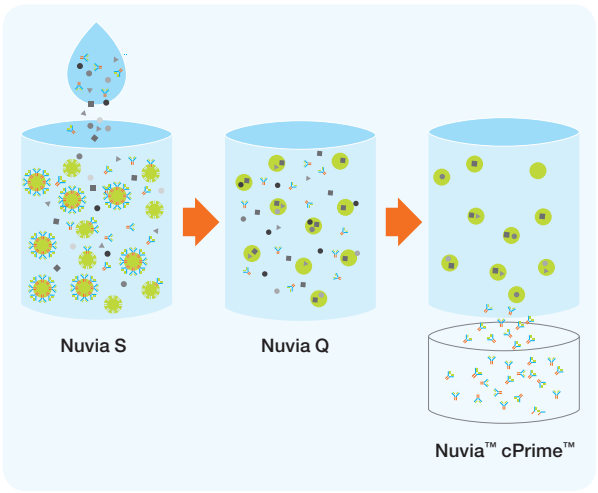
Tissue culture fluid containing mAb G was purified on Nuvia S. HCP and DNA clearance (Table 1) were sufficient to replace affinity chromatography in a three-step process.

Table 1. Impurity clearance on Nuvia S.

	IgG, mg/ml	DNA, ng/ml	HCP, ng/ml
TCF	1.42	176	40,028
Eluate	11.87	211	480
Clearance	–	1.7 logs	2.8 logs

A 10 cm column (0.5 x 10.0 cm) was packed with Nuvia S. The 6x diluted TCF sample was washed in 20 mM sodium acetate, pH 5.0 (buffer A) and eluted in a gradient of buffer A + 1 M NaCl, pH 5.0, at 200 cm/hr.

Purification Workflow



Ordering Information

Catalog #	Description
Prepacked Screening Tools	
732-4701	**Foresight™ Nuvia S Plates**, 2 x 96-well, 20 µl
732-4801	**Foresight Nuvia S RoboColumn Unit**, 200 µl
732-4802	**Foresight Nuvia S RoboColumn Unit**, 600 µl
732-4720	**Foresight Nuvia S Column**, 1 x 1 ml
732-4740	**Foresight Nuvia S Column**, 1 x 5 ml

Bulk Resin	
1560311	**Nuvia S Media**, 25 ml
1560313	**Nuvia S Media**, 100 ml
156-0315	**Nuvia S Media**, 500 ml
156-0317	**Nuvia S Media**, 10 L

Case Studies

The use of Nuvia S for capture in a three-step nonaffinity mAb purification workflow, along with Nuvia Q and Nuvia cPrime, delivers highly purified mAbs with minimal feed conditions. See page 114 for more information.

Nuvia S can be used to fractionate usable proteins from whey in a rapid single-step purification method. Whey proteins play a vital role in the formulation of food supplements due to their numerous nutritional and functional properties. See page 141 for more information.

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CATION EXCHANGE

NUVIA RESINS

Nuvia™ HR-S Resin

FUNCTIONALITY
- High-resolultion polish
- Aggregate removal
- Isoform separation
- Glycoform separation

Nuvia HR-S Resin is a strong cation exchanger, optimized for particle size and pore structure, that provides exceptional resolution and high recovery. The resin is built on Bio-Rad's commercially proven Nuvia base bead that demonstrates fast mass transfer kinetics, excellent flow characteristics, and robust chemical stability against common caustic cleaning protocols. Its excellent scalability gives process developers the confidence that results obtained on the bench will be reproducible for large-scale downstream manufacturing. Nuvia HR-S Resin is the preferred solution for intermediate and final polish applications where process developers face challenging separations of closely related biomolecules.

Benefits

- Purer product from sharper elution profiles
- Higher yields reducing cost of goods
- Consistent results even with variable initial feed conditions
- Chemical stability that withstands repetitive CIP cycles

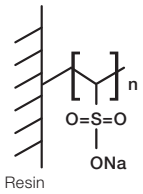


Fig. 1. Nuvia HR-S Resin.

Bead Properties

Property	Description
Type of ion exchanger	Strong cation
Functional group	$-SO_3^-$
Particle size	50 ± 10 µm
Total ionic capacity	100–180 µeq/ml
Dynamic binding capacity	≥70 mg/ml at 300 cm/hr 10% BT capacity determined with 5.0 mg/ml hIgG in 20 mM Na acetate, pH 5.0.
Recommended linear flow rate	50–200 cm/hr
Pressure vs. flow performance	Under 2 bar at flow rate of 200 cm/hr in DI water (20 x 20 cm packed bed, 1.25 compression factor)
Compression factor (settled bed volume/ packed bed volume)	1.20–1.25
pH stability	Short term: 2–14 Long term: 4–13
Shipping solution	20% ethanol
Regeneration	1–2 M NaCl
Sanitization	0.5–1.0 N NaOH
Storage conditions	20% ethanol or 0.1 N NaOH
Chemical stability	
1.0 N NaOH (20°C)	Up to 5 weeks (840 hr)
0.1 N NaOH (20°C)	Up to 5 years
Shelf life	5 years

Binding Capacity

Nuvia HR-S exhibits high binding capacity over a wide operating pH range. The binding capacity of the test protein lactoferrin decreases as the salt concentration increases and optimal binding capacity is seen at lower pH conditions (Figure 2).

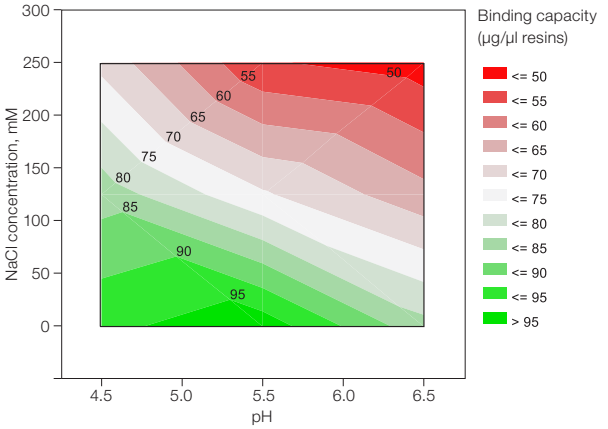


Fig. 2. DoE of lactoferrin binding to Nuvia HR-S at varying pH and NaCl concentrations. Experiments were performed in mini spin columns packed with 400 µl of resin and the dry weight was used to determine the absolute mg of the packed bed. The loading buffer was 20 mM citrate + a variable NaCl concentration. After washing, bound lactoferrin was eluted with buffer containing 1 M NaCl and the binding capacity was calculated based on the amount of lactoferrin eluted divided by the volume of the resin.

Pressure/Flow Performance

Figure 3 shows a linear relationship between pressure and flow up to 200 cm/hr for Nuvia HR-S.

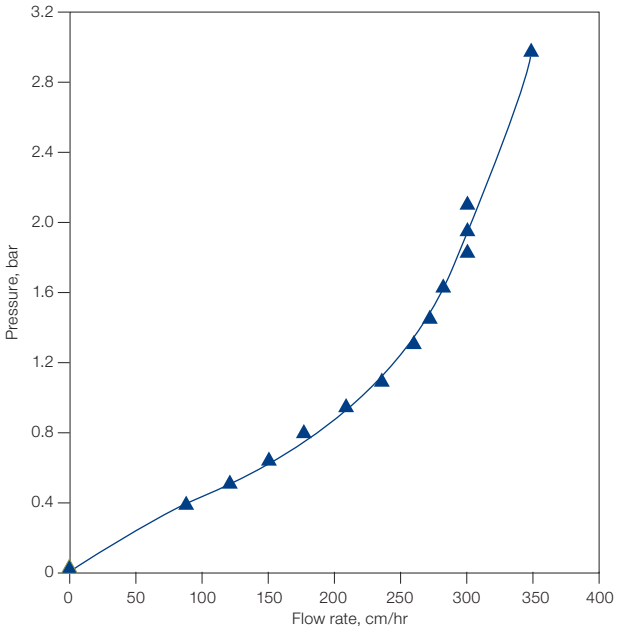


Fig. 3. Nuvia HR-S Resin pressure/flow performance. A 20 cm diameter x 20 cm bed height column was packed to a compression factor of 1.25.

Perfomance Data

Purification of Monomer from Aggregate on Nuvia HR-S

In mAb therapeutics, high molecular weight impurities in the form of aggregates are a key challenge for process developers. Nuvia HR-S delivers the resolution performance that bioprocess scientists need to address this challenge. An example of a high-resolution separation is shown in Figure 4, where a mixture of monomer and aggregate was separated on Nuvia HR-S Resin. Figure 5 shows HPLC-SEC progressive pools collected from the separation of the chromatogram in Figure 4. The data show that even with relatively non-stringent pooling, aggregate levels are significantly reduced. From a starting aggregate concentration of 8.9%, the final eluate pool contained 0.11% residual aggregate with an overall monomer recovery of 82%. Based on the data from Figure 4, an optimized gradient was used for further analysis, as shown in Figure 6.

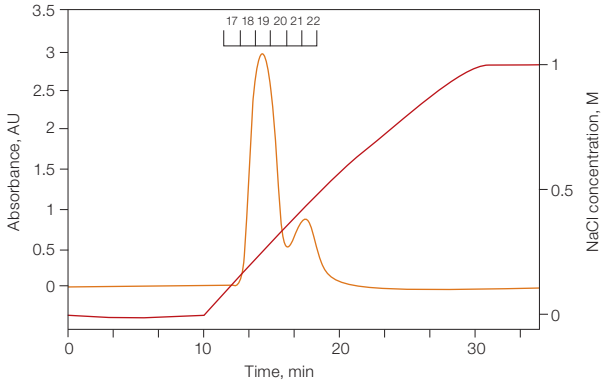


Fig. 4. Separation of aggregates from monomer by Nuvia HR-S before optimization. Absorbance (—); NaCl concentration (—).

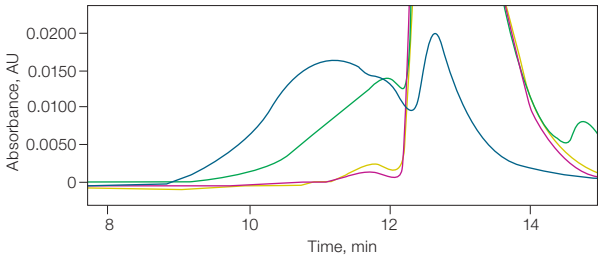
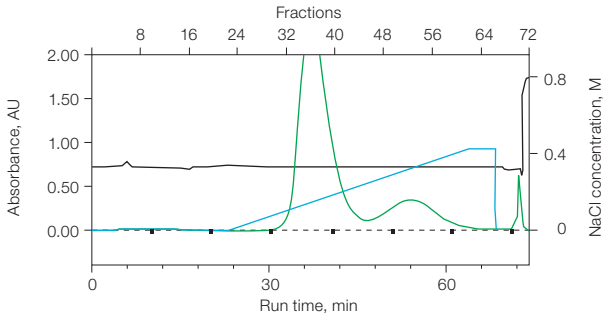


Fig. 5. SEC profiles. Load (—); selected pools (Fr 17+18+19+20) (—) and (Fr 17+18+19) (—); aggregated pool (Fr 21+22) (—).

A



B

Chromatography Condition	Specification
Column	7 x 56 mm Nuvia HR-S Resin packed bed column
Load	20 ml aggregate and monomer mixture at a loading level of 46 mg/ml
Flow rate	150 cm/hr
Equilibration	40 mM Na acetate, pH 5.0
Gradient elution	40 mM Na acetate, 0–0.4 M NaCl, pH 5.0

Fig. 6. Monoclonal antibody separation on Nuvia HR-S Resin. **A**, chromatogram of the purification of monomer from aggregate; **B**, chromatography conditions. A_{280} (—); pH (—); gradient elution (—).

Comparitive Aggregate Content and Monomer Recovery Using Nuvia HR-S

Nuvia HR-S Resin was compared to a commercially available small particle size CEX resin (Resin 1) to determine the aggregate content and monomer recovery as fractions were progressively pooled during elution. Nuvia HR-S delivered a final aggregate content of <0.3% and a recovery of >80%, while the recovery using agarose-based Resin 1 was <70% for the same final aggregate content (Figure 7).

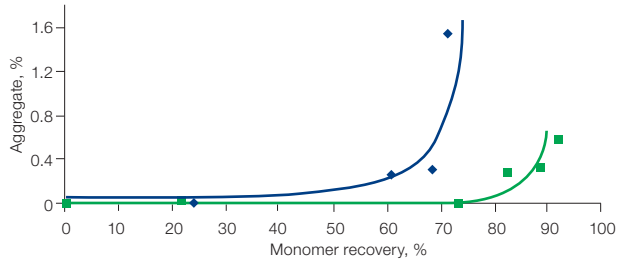
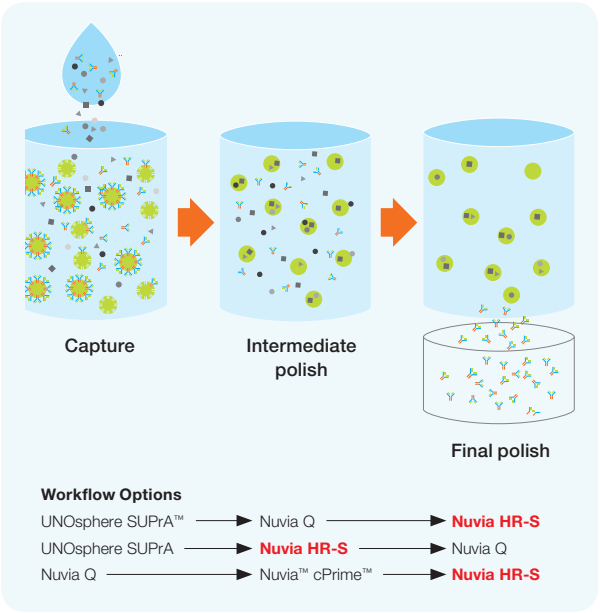


Fig. 7. Performance of Nuvia HR-S (■) vs. Resin 1 (◆).

Purification Workflow



Case Studies

Nuvia HR-S is productive tool for manufacturers to produce consistent biotherapeutic products as the high-resolution CEX resin can resolve closely related species differing by one or a few deamidiation events. See page 120 for more information.

Nuvia HR-S used during final polishing effectively removes aggregates and other impurities and provides higher percent recovery compared to agarose-based small particle CEX resins. See page 128 for more information.

Ordering Information

Catalog #	Description
Prepacked Screening Tools	
732-4707	**Foresight™ Nuvia HR-S Plate**, 20 µl
732-4831	**Foresight Nuvia HR-S RoboColumn Unit**, 200 µl
732-4832	**Foresight Nuvia HR-S RoboColumn Unit**, 600 µl
732-4723	**Foresight Nuvia HR-S Column**, 1 ml
732-4743	**Foresight Nuvia HR-S Column**, 5 ml

Bulk Resin	
1560511	**Nuvia HR-S Media**, 25 ml
1560513	**Nuvia HR-S Media**, 100 ml
156-0515	**Nuvia HR-S Media**, 500 ml
156-0517	**Nuvia HR-S Media**, 10 L

ANION EXCHANGE

NUVIA RESINS

Nuvia™ Q Resin

FUNCTIONALITY
- High capacity
- Selectivity
- DNA removal
- Viral clearance
- Endotoxin clearance

Nuvia Q is an ultra high capacity next-generation AEX resin built on the robust, industry-proven Nuvia base matrix. Nuvia Q contains quaternary amine groups attached to propietary polymeric surface extenders. Nuvia Q Resin delivers high binding capacity across a range of pH and flow rates, providing wide experimental design space for process developers. Its exceptional binding capacity makes it the anion exchanger of choice for biopharmaceuticals as upstream processes deliver higher concentrations of feed titers. Nuvia Q also delivers excellent performance for polishing applications and can significantly improve productivity while contributing to reduced capital costs, space requirements, and cycle time for downstream biotherapeutic purification. The unique resin design gives Nuvia Q best-in-class dynamic binding capacity.

Benefits

- Ultra-high binding capacity
- Effective resolution of biomolecules from crude feedstreams
- Rapid mass transfer kinetics
- Robust performance at high flow rates
- Excellent clearance of host cell DNA in flowthrough

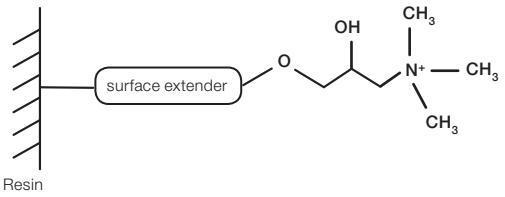


Fig. 1. Nuvia Q Resin.

Bead Properties

Property	Description
Type of ion exchanger	Strong anion
Functional group	$-N^+(CH_3)_3$
Particle size	85 ± 15 µm
Total ionic capacity	100–170 µeq/ml
Dynamic binding capacity	>170 mg/ml at 300 cm/hr 10% BT capacity determined with 5 mg/ml BSA in 20 mM Tris-HCl, pH 8.5
Recommended linear flow rate	50–600 cm/hr
Pressure vs. flow performance	Under 3 bar up to 500 cm/hr in DI water
Compression factor (settled bed volume/ packed bed volume)	1.10–1.15
pH stability	Short term: 2–14 Long term: 4–12
Shipping solution	20% ethanol + 0.1 M NaCl
Regeneration	1–2 M NaCl
Sanitization	0.5–1.0 N NaOH
Storage conditions	20% ethanol or 0.01 N NaOH
Chemical stability 1.0 N NaOH (20°C) 0.01 N NaOH (20°C)	 Up to 1 week Up to 5 years
Shelf life	5 years

Binding Capacity

Nuvia Q Resin is designed to meet the demanding pressure-flow requirements of downstream processes. With strong mechanical properties and optimized bead size distribution, Nuvia Q consistently delivers high binding capacity at fast linear flow velocities (Figure 2), allowing users the advantage of increased productivity without compromising binding capacity.

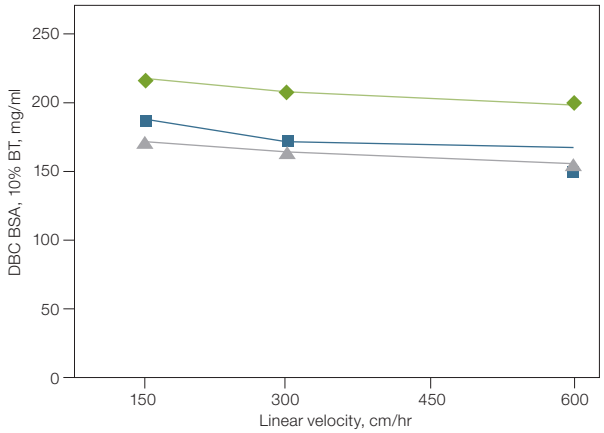


Fig. 2. DBC vs. flow velocity of Nuvia Q Resin. Each 1.1 cm column was packed to a 10.6 cm bed height with Nuvia Q, agarose Q, or polymeric Q resin. BSA (5 mg/ml) in 20 mM Tris-HCl, pH 8.5, was loaded onto each column until 10% BT was observed. Nuvia Q Resin (◆); agarose Q resin (■); polymeric Q resin (▲).

Pressure/Flow Performance

Figure 3 shows the effect of flow rate on Nuvia Q Resin backpressure.

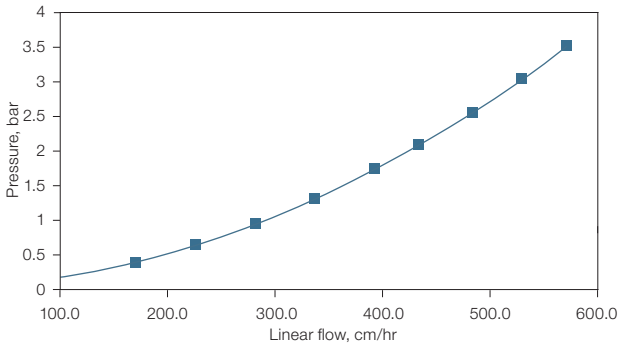


Fig. 3. Nuvia Q Resin pressure/flow performance. A 20 cm diameter x 20 cm bed height column was packed to a compression factor of 1.10.

Perfomance Data

Nuvia Q Resin exhibits excellent baseline resolution of biomolecules with similar pls. Figure 4 shows the purification of α-lactalbumin (ALA) and β-lactoglobulin (BLG), the major proteins of whey, accounting for approximately 75% of the total mass of crude whey. The two major peaks were resolved using a segmented NaCl gradient. SDS-PAGE analysis (Figure 5) of the fractions showed two protein peaks, one containing ALA (Peak 1) and the other containing BLG (Peak 2), with pls of 4.7–5.1 and 5.2, respectively. Purities of the two purified proteins were estimated by densitometry to be at least 85%.

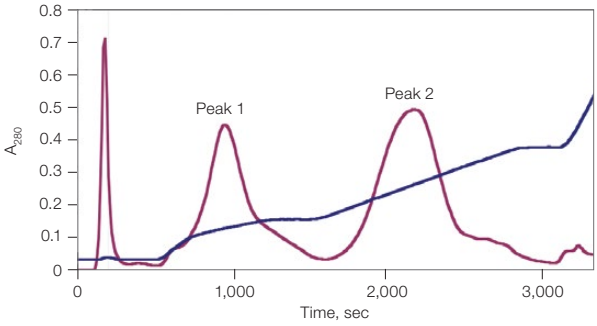


Fig. 4. Purification of whey protein on Nuvia Q Resin. Crude whey feed stock (8 mg) was loaded on a 7 x 27 mm column in 0.02 M sodium phosphate buffer, pH 6.0. The samples were eluted using a segmented gradient of 0–0.1 M NaCl, 0.1–0.3 M NaCl, and 0.3–0.9 M NaCl. Each fraction was 2 ml and analyzed at OD 280. A_{280} (—); conductivity (—).

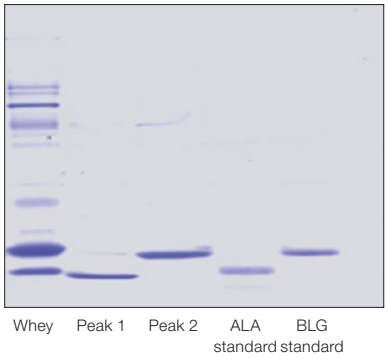
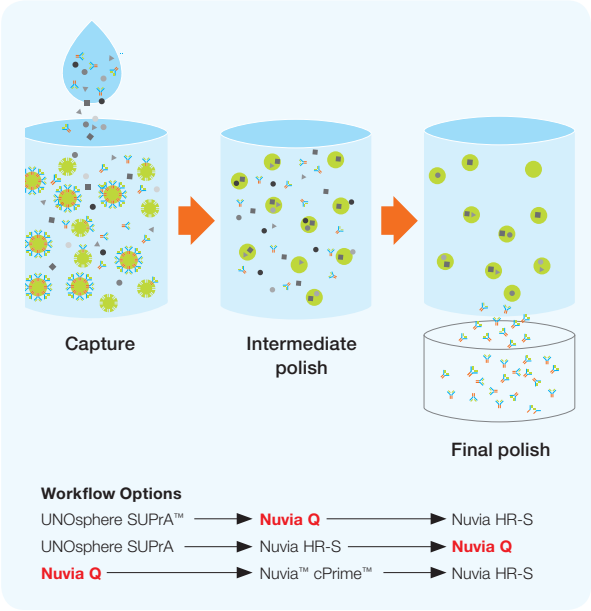


Fig. 5. Analysis of whey purified on Nuvia Q Resin. Fractions collected from the chromatography run shown in Figure 4 were separated by SDS-PAGE.

Purification Workflow



Ordering Information

Catalog #	Description
Prepacked Screening Tools	
732-4703	**Foresight™ Nuvia Q Plates**, 2 x 96-well, 20 µl
732-4804	**Foresight Nuvia Q RoboColumn Unit**, 200 µl
732-4805	**Foresight Nuvia Q RoboColumn Unit**, 600 µl
732-4721	**Foresight Nuvia Q Column**, 1 x 1 ml
732-4741	**Foresight Nuvia Q Column**, 1 x 5 ml

Bulk Resin

1560411	**Nuvia Q Media**, 25 ml
1560413	**Nuvia Q Media**, 100 ml
156-0415	**Nuvia Q Media**, 500 ml
156-0417	**Nuvia Q Media**, 10 L

Case Studies

The use of Nuvia Q for intermediate polishing in a three-step nonaffinity mAb purification workflow, along with Nuvia S and Nuvia cPrime, delivers highly purified mAbs with minimal feed conditions. See page 114 for more information.

An efficient, readily scalable two-step cGMP-ready adenovirus purification process using Nuvia cPrime for mass capture followed by Nuvia Q for final polishing delivers increased total protein recovery and purity. See page 123 for more information.

Nuvia Q can be used to fractionate usable proteins from whey in a rapid single-step purification method. Whey proteins play a vital role in the formulation of food supplements due to their numerous nutritional and functional properties. See page 141 for more information.


MIXED MODE

NUVIA RESINS

Nuvia™ cPrime™ Resin

FUNCTIONALITY
- Unique selectivity
- Nonaffinity capture
- Salt tolerant
- L chain fragment removal
- Aggregate removal

The Nuvia bead is built on a polymeric base matrix that delivers low backpressure at high flow rates. Nuvia cPrime is effective for the purification of established therapeutic proteins as well as the increasingly diverse new constructs that are in development, many of which lack an affinity handle. Salt- and pH-sensitive proteins with a high propensity for aggregation and/or degradation can be effectively purified using simplified methods.

Nuvia cPrime Resin is designed with a mixed-mode ligand that provides a unique balance between hydrophobic and charged characteristics. The ligand structure also provides an opportunity for hydrogen-bonding interactions. Importantly, the balance of weak acid and hydrophobic components is optimized to allow for straightforward method development and predictable behavior during binding and elution.

Benefits

- Unique selectivity
- Salt tolerance
- Simple method development
- Large design space for binding and elution
- High recovery
- Mechanical and chemical stability

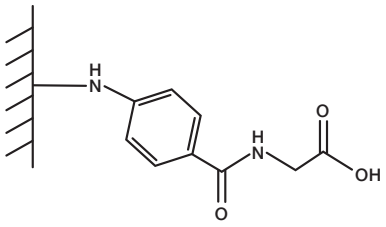


Fig. 1. Mixed-mode ligand for Nuvia cPrime Resin.

Bead Properties

Property	Description
Type of ion exchanger	Hydrophobic weak cation exchange
Functional group	See Figure 1
Particle size	70 ± 10 µm
Ligand density	123 ± 20 µeq/ml
Dynamic binding capacity	>40 mg hIgG/ml (at 10% BT, 300 cm/hr) >60 mg lactoferrin/ml
Recommended linear flow rate	50–600 cm/hr
Pressure vs. flow performance	Under 2 bar at flow rate of 600 cm/hr in DI water (20 x 20 cm packed bed, 1.17 compression factor)
Compression factor (settled bed volume/ packed bed volume)	1.15–1.20
pH stability	Short term: 3–14 Long term: 4–13
Shipping solution	20% ethanol, 30 mM Na_2SO_3
Regeneration	1 N NaOH
Sanitization	1 N NaOH
Storage conditions	0.1 M NaOH
Chemical stability	1.0 N NaOH, 8 M urea, 6 M guanidine-HCl, 6 M potassium thiocyanate, 3 M NaCl, 1% Triton X-100, 2% SDS + 0.25 M NaCl, 20% ethanol, 70% ethanol, 30% isopropyl alcohol
Shelf life	5 years

Binding Capacity

Nuvia cPrime is designed for versatile capture and high recovery at high flow rates (Figure 2) and across a wide range of salt concentrations and pH (Figure 3). These properties, summarized in Table 1, may allow for direct loading without the need for dilution. Integrating a Nuvia cPrime step into a multicolumn process is operationally simple.

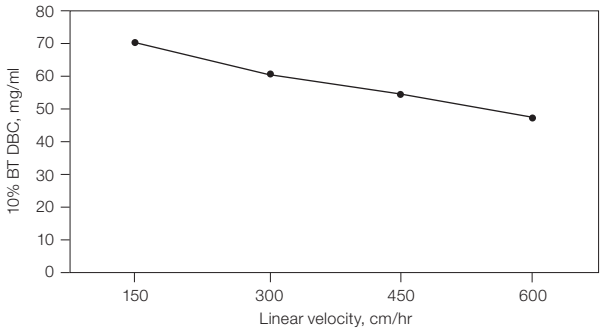


Fig. 2. DBC vs. linear velocity of Nuvia cPrime Resin. A 1.1 x 9.6 cm column was loaded with 5.25 mg/ml lactoferrin in 20 mM NaOAc + 150 mM NaCl, pH 4.5, until 10% BT was observed.

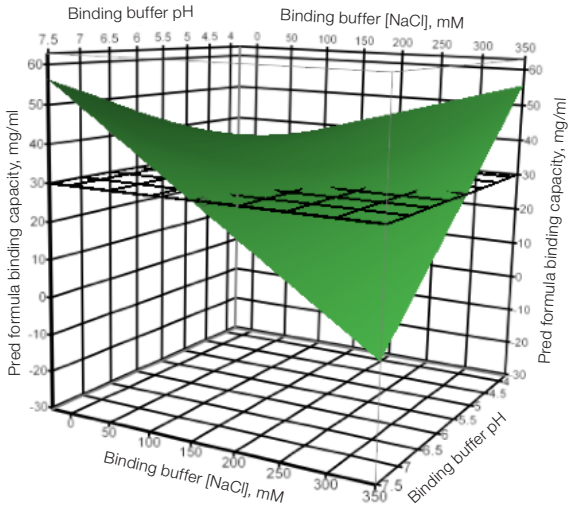


Fig. 3. Predicted binding capacity of Nuvia cPrime for mAb2 at varying pH and NaCl concentrations.

Table 1. mAb X binding capacity and recovery as a function of Nuvia cPrime flow rate.

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

Pressure/Flow Performance

Nuvia cPrime is built on a porous polymeric base matrix that delivers low backpressure at high flow rates (Figure 4). It is also chemically and mechanically stable. Fast mass transfer dynamics ensure efficient chromatography at high flow, making Nuvia cPrime Resin an operationally superior choice for commercial-scale applications.

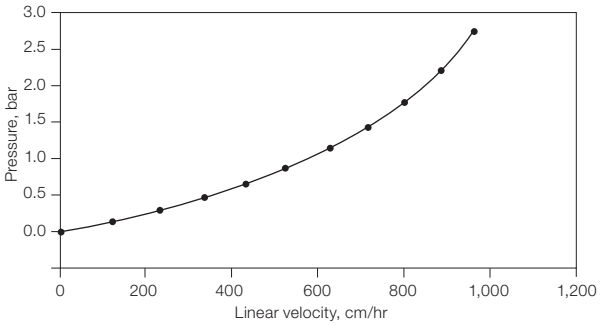


Fig. 4. Nuvia cPrime displays low backpressure at high flow rates. Flow performance of Nuvia cPrime Resin in a Bio-Rad® InPlace™ Column. A 20 x 20 cm column with 1.17 axial compression was used.

Performance Data

The balance between hydrophobicity, charged interaction, and the highly hydrophilic base matrix of Nuvia cPrime empowers method developers with new ways to directly exploit various modes of interaction. This allows purification of challenging or sensitive proteins or separation of closely related protein species, such as isoforms and variants from posttranslational modifications and product aggregates and fragments.

Partially purified mAb1 from a Nuvia Q polishing step can be loaded onto a Nuvia cPrime Column following a simple pH adjustment to positively charge mAb1, without extensive dilution or buffer exchange for conductivity reduction. A high concentration of salts, such as $(NH_4)_2SO_4$, was not needed for promoting the binding of target protein either. Such behavior makes Nuvia cPrime distinctive from conventional hydrophobic interaction resins, which require high salt levels for effective protein binding. Under the selected condition, only the desired full-length mAb1 was retained by the column (Figure 5A). A 25-kD L-chain fragment of this antibody appeared in the column flow-through fractions, as visualized on SDS-PAGE (Figure 5B) and identified by mass spectrometry (data not shown) and HPLC-SEC (Figure 5C).

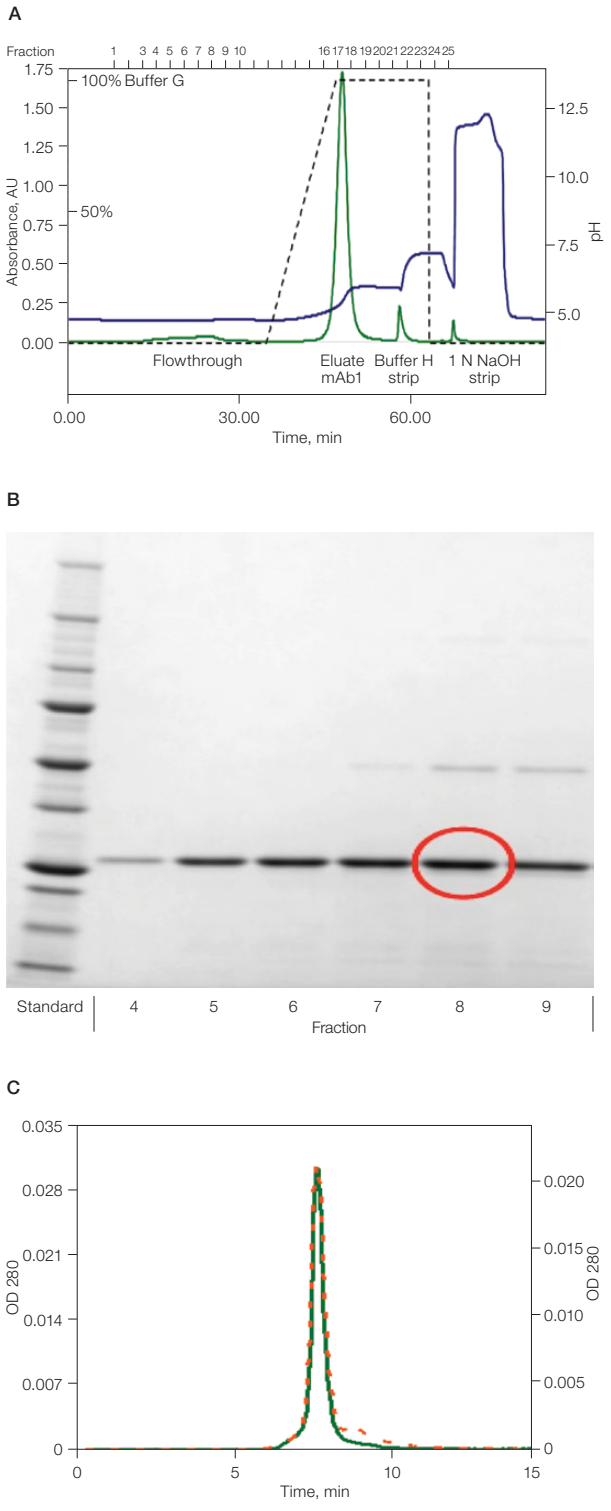
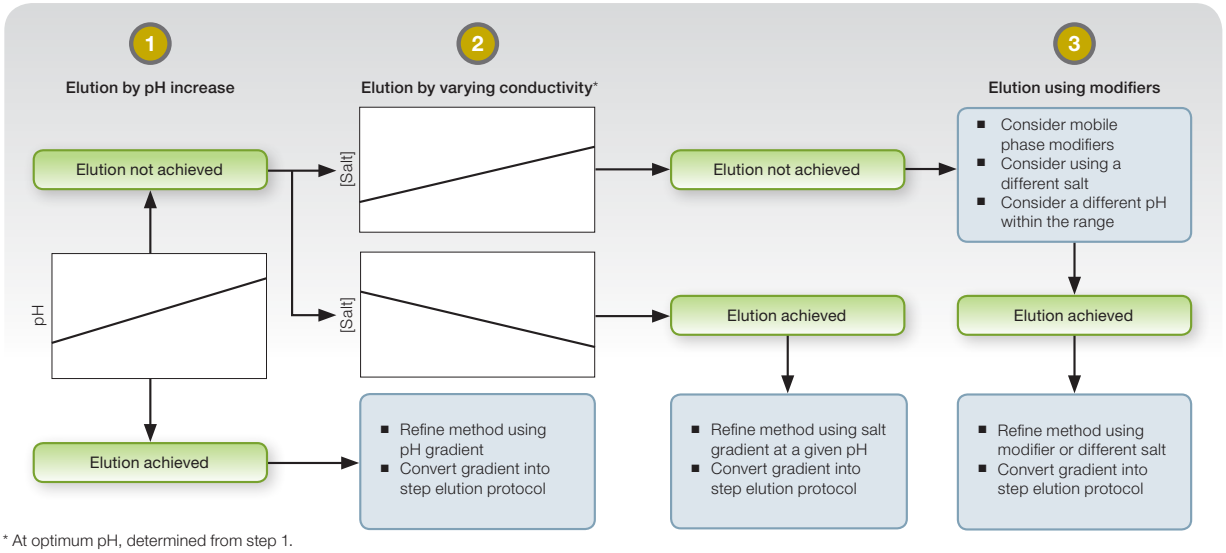


Fig. 5. Final polishing purification of mAb1 using Nuvia cPrime. **A**, chromatogram of mAb1 purification on a Nuvia cPrime Column. OD 280 (—); pH (—); percentage of buffer G (- - -). mAb1 eluted from the column (fractions 17–19) was collected and subjected to purity analysis. **B**, SDS-PAGE analysis of column flowthrough (fractions 4–9); Precision Plus Protein™ Unstained Standards were used. A major protein impurity was excised from the gel (circled in red) and analyzed by MALDI-TOF mass spectrometry. **C**, HPLC-SEC comparison of pooled Nuvia Q fractions (- - -) and pooled Nuvia cPrime fractions (—).

Process Workflow

The mixed-mode nature of the Nuvia cPrime ligand and its associated range of interactions allows for a directed and intuitive approach to method development and process optimization (Figure 6). Alternatively, a simple DoE exercise will quickly guide developers to optimum loading, wash, and elution conditions afforded by the resin's large design space.



* At optimum pH, determined from step 1.

Fig. 6. Recommended approach to method development.

Ordering Information

Catalog #	Description
Prepacked Screening Tools	
732-4705	**Foresight™ Nuvia cPrime Plates**, 2 x 96-well, 20 µl
732-4807	**Foresight Nuvia cPrime RoboColumn Unit**, 200 µl
732-4808	**Foresight Nuvia cPrime RoboColumn Unit**, 600 µl
732-4722	**Foresight Nuvia cPrime Column**, 1 ml
732-4742	**Foresight Nuvia cPrime Column**, 5 ml

Bulk Resin	
1563401	**Nuvia cPrime Media**, 25 ml
1563402	**Nuvia cPrime Media**, 100 ml
156-3403	**Nuvia cPrime Media**, 500 ml
156-3404	**Nuvia cPrime Media**, 1 L
156-3405	**Nuvia cPrime Media**, 5 L
156-3406	**Nuvia cPrime Media**, 10 L

Case Studies

The use of Nuvia cPrime for polishing in a three-step nonaffinity mAb purification workflow, along with Nuvia S and Nuvia Q, delivers highly purified mAbs with minimal feed conditions. See page 114 for more information.

An efficient, readily scalable two-step cGMP-ready adenovirus purification process using Nuvia cPrime for mass capture followed by Nuvia Q for polishing achieved a tenfold reduction in processing volume and a reduction in feedstream contaminants. See page 123 for more information.

Notes

UNOsphere™ Resins

IEX resins versatile in both capacity and resolution provide a cost-effective solution

Composition and Properties

Polymer	Properties	Derivatives
▪ Macroporous hydrophilic polymer produced from water soluble acrylamido and vinylic monomers ▪ Controlled one-step aqueous polymerization incorporates the functional monomer during polymerization, promoting superior lot-to-lot reproducibility	▪ Average particle size range from 80 to 120 µm ▪ Pore structure can be gel phase or open pore depending on chemistry ▪ Constant DBC over a wide range of flow rates (exceeding 500 cm/hr) ▪ Fast mass-transfer due to high porosity ▪ Multimodal functionality	▪ S and Q (strong) ▪ Affinity (Protein A)

Advantages

Chromatographer	Engineer	Economic buyer
▪ Balance between capacity and resolution properties ▪ Wider design space over a range of flow rates ▪ May be able to exploit carboxyl content to form a pH gradient with UNOsphere S	▪ Minimal tailing resulting in lower elution volumes ▪ Chemical and mechanical stability ▪ Lot-to-lot reproducibility ▪ Easy to pack	▪ Withstands multiple CIP cycles ▪ Robust chemical stability

INTRODUCTION

UNOsphere IEX Resins are unique compared to most chromatography resins, which are manufactured by first producing a base polymer that is then converted through one or more chemical reactions to a functional ion exchange derivative. They are produced in a controlled single-step polymerization using matrix, crosslinker, and functional monomers. During the formation of spherical beads, quaternary amine or sulfonic acid IEX groups are incorporated directly into the polymer. UNOsphere Q and UNOsphere S Resins are produced from water-soluble acrylamido and vinylic monomers, and therefore are hydrophilic and exhibit low nonspecific adsorption. The average particle size for UNOsphere ion exchangers is 80–120 µm. UNOsphere IEX Resins have excellent pressure-flow properties. Protein binding capacity is well maintained over a broad range of flow rates ≥500 cm/hr. These ion exchangers have broad applications and perform well in capture, intermediate, and polishing purification steps. UNOsphere S selectivity is distinct from Nuvia HR-S, hence screening both resins is recommended for cation polish steps.


CATION EXCHANGE

UNOsphere RESINS

UNOsphere™ S Resin

FUNCTIONALITY
- Unique selectivity
- Isoform separation
- Viral clearance

UNOsphere S CEX Resin is designed to meet the increasing demands of the biopharmaceutical industry. UNOsphere S, a hydrophilic polymeric resin based on a single-step polymerization process, delivers high productivity through high binding capacity and low backpressure and is selected for the separation of proteins, nucleic acids, viruses, plasmids, and other biomolecules. The single-step polymerization process produces a resin unsurpassed in quality and batch-to-batch reproducibility. In addition, this resin possess a unique ability to self-generate a pH gradient during certain elution schemes. This gives UNOsphere S resolving power not attainable with other S-type resins.

Benefits

- Efficient capture of biopharmaceutical molecules from crude feedstreams
- High binding capacities at fast linear velocities
- Hydrophilic polymeric beads engineered for high mechanical stability and low backpressures
- Robust polymers designed to withstand repetitive CIP cycles
- Large open pore structure for fast mass transfer

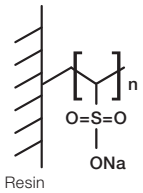


Fig. 1. UNOsphere S Resin.

Bead Properties

Property	Description
Type of ion exchanger	Strong cation
Functional group	$-SO_3^-$
Particle size	80 µm
Total ionic capacity	269 ± 50 µeq/ml
Dynamic binding capacity	60 mg IgG/ml at 150 cm/hr 10% BT capacity determined with 4.5 mg/ml hIgG in 1.1 x 10 cm column
Recommended linear flow rate	50–300 cm/hr
Pressure vs. flow performance	Under 2.0 bar at flow rate of 1,200 cm/hr in DI water (20 x 20 cm packed bed, 1.17 compression factor)
Compression factor (settled bed volume/ packed bed volume)	1.15–1.20
pH stability	1–14
Shipping solution	20% ethanol or 0.1 M NaCl
Regeneration	1–2 M NaCl
Sanitization	0.5–1.0 N NaOH
Storage conditions	20% ethanol or 0.1 N NaOH
Chemical stability	
1.0 M NaOH (20°C)	Up to 2,000 hr
1.0 M HCl (20°C)	Up to 200 hr**
	** Data derived under accelerated conditions at 60°C
Shelf life	5 years

Binding Capacity

The mean particle size of UNOsphere S Resin is 80 µm, which generates a low backpressure. The highly macroporous nature of UNOsphere S Resins provides high binding capacities that range from 40–60 mg IgG/ml resin in the linear velocity range of 150–600 cm/hr (Figure 2).

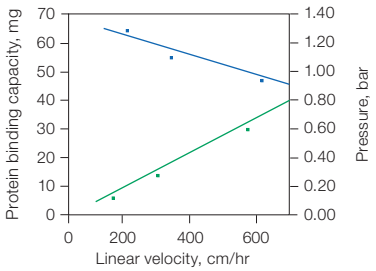


Fig. 2. Binding and backpressure properties of UNOsphere S Resin. Column size, 1.1 × 20 cm; sample, 2 mg/ml hIgG; buffer, 50 mM sodium acetate, pH 5.0. Backpressure (—); 10% BT capacity (—).

Pressure/Flow Performance

UNOsphere S Resins were designed to achieve the highest productivity (grams of drug per operational hour per liter of media) possible. UNOsphere Resins can be run at the highest linear velocities and loading capacities allowed by the column and chromatography system. Figure 3 illustrates the pressure/flow performance for UNOsphere S Resin.

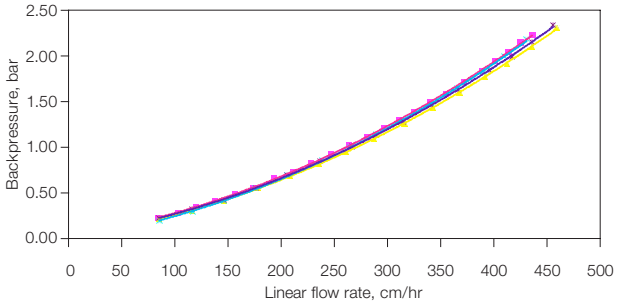


Fig. 3. Normalized pressure/flow curves for comparison of running buffers on UNOsphere S. 10 mM sodium acetate, 150 mM NaCl, pH 5.0 (—); 0.1 M NaCl (—); 1.0 M NaCl (—); PBS, pH 7.0 (—).

Performance Data

UNOsphere S Resins provide high-efficiency capture of mAbs from crude feedstreams. Murine IgG1 (6.6 mg) was captured and eluted from a 2 ml UNOsphere S Column (Figure 4); assays of the load and eluate demonstrated a recovery of 97%. No antibody was detected in the flowthrough. See bulletin 2669 for complete information.

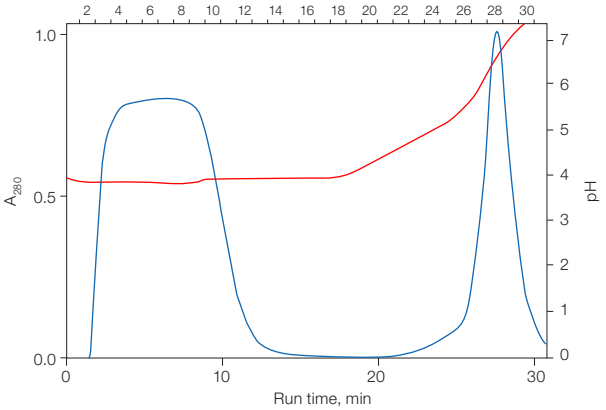
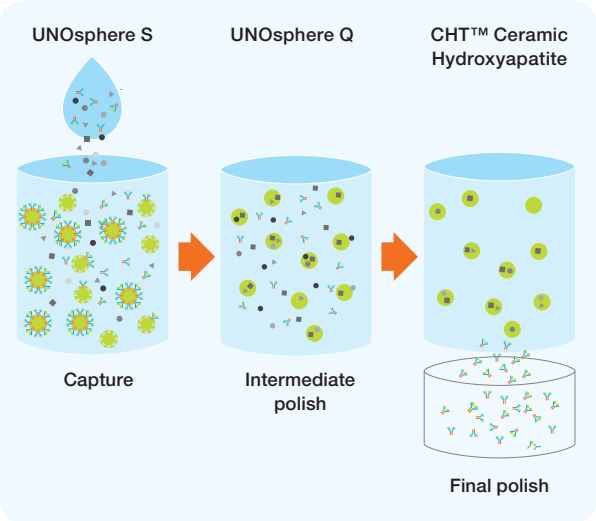


Fig. 4. Purification of murine IgG1 on a UNOsphere S Column. Column size, 0.5 × 10 cm (2 ml); sample, 15 ml (6.6 mg) of murine IgG1-conditioned medium. The sample was loaded onto the column in 20 mM citrate-phosphate buffer, pH 4.0, washed, and eluted in a linear gradient of 0–100% 20 mM citrate-phosphate, pH 8.0, in 10 CV at a flow rate of 2.0 ml/min (600 cm/hr). Each fraction was 2.0 ml. A_{280} (—); buffer pH (—).

Purification Workflow



Ordering Information

Catalog #	Description
Prepacked Screening Tools	
732-4710	**Foresight™ UNOsphere S Plates**, 2 x 96-well, 20 µl
732-4813	**Foresight UNOsphere S RoboColumn Unit**, 200 µl
732-4814	**Foresight UNOsphere S RoboColumn Unit**, 600 µl
732-4730	**Foresight UNOsphere S Column**, 1 x 1 ml
732-4750	**Foresight UNOsphere S Column**, 1 x 5 ml

Bulk Resin	
1560111	**UNOsphere S Support**, 25 ml
1560113	**UNOsphere S Support**, 100 ml
156-0115	**UNOsphere S Support**, 500 ml
156-0117	**UNOsphere S Support**, 10 L

UNOsphere RESINS

UNOsphere™ Q Resin

FUNCTIONALITY
- DNA removal
- Viral clearance

UNOsphere Q is a high-capacity, high-throughput AEX resin, based on acrylamido and vinylic monomers, designed for process chromatography. The resin was designed with large-diameter pores and a large surface area to maximize capture speed, macromolecule capacity, recovery, and productivity. UNOsphere Q contains a low-density gel phase within a matrix of denser polymer aggregates, which provides high binding capacities of 125–180 mg BSA per ml of resin in the linear velocity range of 150–1,200 cm/hr. UNOsphere Q is highly productive compared to other process resins having similar functional groups when operated at a constant pressure.

Benefits

- Efficient capture of impurities in flow-through mode
- Fast linear velocities, maximizing productivity
- High binding capacities due to its large pore size
- Robust polymers designed to withstand repetitive CIP cycles

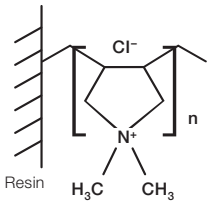


Fig. 1. UNOsphere Q Resin.

Bead Properties

Property	Description
Type of ion exchanger	Strong anion
Functional group	$-N^{+}(CH_3)_3$
Particle size	120 µm
Total ionic capacity	120 µeq/ml
Dynamic binding capacity	≥180 mg/ml at 150 cm/hr ≥125 mg/ml at 600 cm/hr 10% BT capacity determined with 2.0 mg/ml BSA in 1.1 x 10 cm column
Recommended linear flow rate	50–300 cm/hr
Pressure vs. flow performance	Under 2 bar at flow rate of 1,200 cm/hr in DI water (20 x 20 cm packed bed, 1.20 compression factor)
Compression factor (settled bed volume/ packed bed volume)	1.15 –1.20
pH stability	1–14
Shipping solution	20% ethanol or 0.1 M NaCl
Regeneration	1–2 M NaCl
Sanitization	0.5–1.0 N NaOH
Storage conditions	20% ethanol or 0.1 M NaOH
Chemical stability	
1.0 M NaOH (20°C)	Up to 10,000 hr
1.0 M HCl (20°C)	Up to 200 hr
Shelf life	5 years

Binding Capacity

Most production chromatography systems have maximum pressure limits of 3 bar. The mean particle size of UNOsphere Q Resin is 120 µm, which generates a low backpressure (Figure 2). Increasing flow rates have minimal impact on binding capacity due to the excellent mass transfer properties of UNOsphere Q.

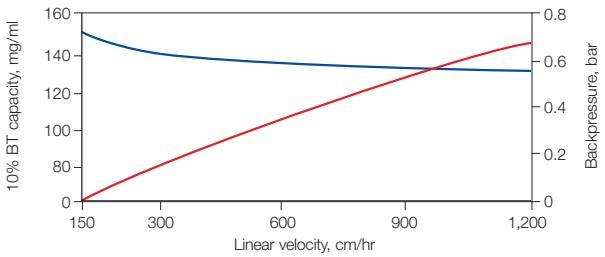


Fig. 2. Binding and backpressure properties of UNOsphere Q Resin. A 5 mg/ml sample of BSA in 10 mM Tris, pH 8.5, was loaded onto a 1.1 x 20 cm column. Backpressure (—); 10% BT capacity (—).

Pressure/Flow Performance

The high productivity exhibited by UNOsphere Q Resin compared to other commercially available resins is probably due in part to its open architecture and low backpressure at high flow rates (Figure 3).

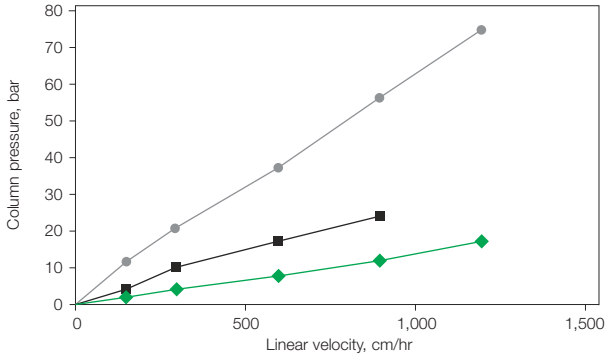


Fig. 3. Pressure/flow comparison for AEX resins. Conditions were as in Table 1. The Q Sepharose FF was run above the manufacturer's recommended 600 cm/hr. UNOsphere Q (-◆-); Q Sepharose FF (-■-); Fractogel EMD TMAE (M) (-●-).

Performance Data

An inverse relationship exists between the DBC and productivity, and the latter continues to increase even at 1,200 cm/hr (Figure 4). Productivity for UNOsphere Q Resin compares favorably at 1 bar constant pressure with that of other process resins (Figure 5 and Table 1).

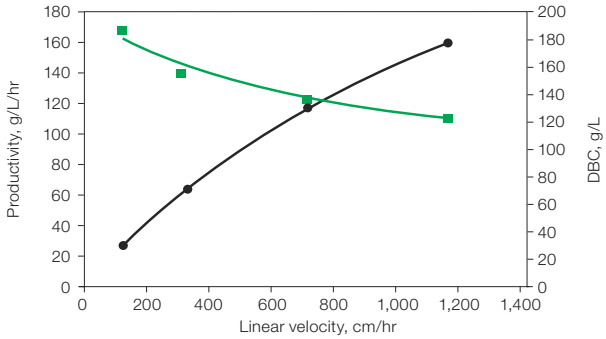


Fig. 4. Protein binding capacity (—) and productivity (—) of UNOsphere Q Resin. Conditions were as in Table 1.

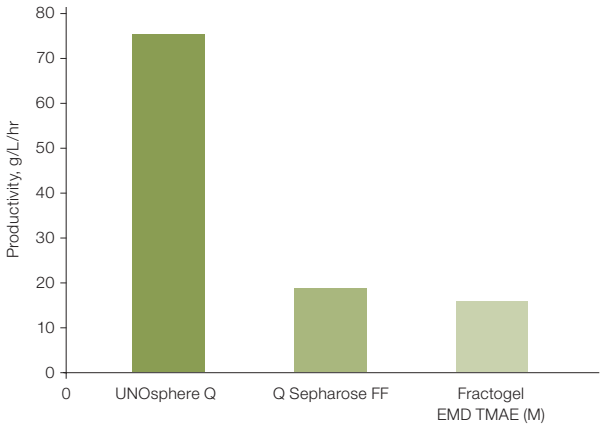


Fig. 5. UNOsphere Q productivity comparison. Conditions were as in Table 1.

Table 1. Comparison of properties of UNOsphere Q, Q Sepharose, and Fractogel TMAE Resins.

Support	Linear Velocity, cm/hr	Recovery, %	BSA Binding Capacity, g/L	Process Time, hr	Productivity, g/L/hr
UNOsphere Q	615	100.0	120.0	1.58	75.0
Q Sepharose FF	300	99.0	23.0	1.19	19.0
Fractogel EMD TMAE (M)	105	99.0	82.0	5.04	16.0

Properties were evaluated on a 1.1 x 20 cm (20 ml) column equilibrated with 10 mM Tris buffer, pH 8.5 (buffer A). BSA (5.0 mg/ml in buffer A) was loaded until 10% BT occurred. Elution was performed with buffer A containing 0.5 M NaCl.

High concentrations of salt or chaotropic agents are often required for the chromatography of inclusion body proteins and column regeneration, among other things. Table 2 shows that addition of these chemicals does not increase column pressures at high flow rates.

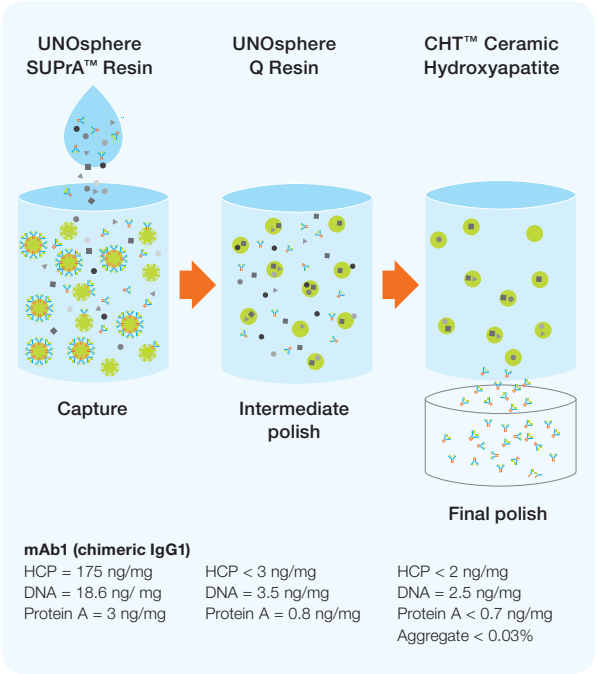
Table 2. Column pressure in various test solvents over a range of linear velocities.*

	Pressure (psi) at Given Linear Velocity (cm/hr)				
Solvent	150	300	600	900	1,200
20 mM Tris, pH 8.5	2	2	4	8	9
1 M NaCl	2	3	6	9	11
1 N NaOH	3	3	6	9	10
6 M guanidine-HCl	7	9	15	22	ND**
8 M urea	6	14	28	43	52

* Resin was suspended in 1 M NaCl and packed into a 1.1 x 20 cm column at 1,200 cm/hr. The column was equilibrated with 20 mM Tris buffer, pH 8.5, then with the test solvent, and run at the velocities indicated. Between tests, the column was re-equilibrated with Tris buffer.

** ND, not determined

Purification Workflow



Ordering Information

Catalog #	Description
Prepacked Screening Tools	
732-4714	**Foresight™ UNOsphere Q Plates**, 20 µl
732-4819	**Foresight UNOsphere Q RoboColumn Unit**, 200 µl
732-4820	**Foresight UNOsphere Q RoboColumn Unit**, 600 µl
732-4732	**Foresight UNOsphere Q Column**, 1 ml
732-4752	**Foresight UNOsphere Q Column**, 5 ml

Bulk Resin	
1560101	**UNOsphere Q Support**, 25 ml
1560103	**UNOsphere Q Support**, 100 ml
156-0105	**UNOsphere Q Support**, 500 ml
156-0107	**UNOsphere Q Support**, 10 L

Case Studies

The use of UNOsphere Q for intermediate polishing in a three-step mAb purification process, along with UNOsphere SUPrA and CHT, provides a platform for superior purity and recovery. See page 110 for more information.

UNOsphere RESINS

UNOsphere SUPrA™ Resin

FUNCTIONALITY
- Fc fusion protein purification
- Mild elution conditions
- Low elution volumes

UNOsphere SUPrA Affinity Resin is designed for process-scale purification of monoclonal and polyclonal antibodies. This affinity resin is built on the proven UNOsphere™ matrix for predictable performance over a wide range of antibody concentrations. With UNOsphere SUPrA Resin, higher bed heights can be used to increase residence time without excessive pressure increases, providing developers with a large window of operational freedom. UNOsphere SUPrA typically provide >95% recovery of target antibodies.

Benefits

- Reliable performance and high productivity
- Superior flow properties
- Narrow elution profiles that reduce the volume of buffer required
- Higher bed heights for increased residence time
- Elution of mAbs at higher pH for increased product stability
- High DBC for mAbs, even with low-titer feedstreams

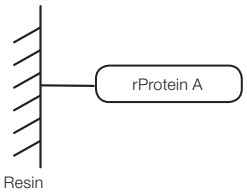


Fig. 1. UNOsphere SUPrA Resin.

Bead Properties

Property	Description
Ligand	Recombinant Protein A
Particle size	53–61 µm
Total ligand density	10 mg/ml
Dynamic binding capacity	30 ± 3 mg/ml 10% BT capacity determined with 1.0 mg/ml polyclonal hIgG in 1.1 x 10 cm column
Recommended linear flow rate	100–600 cm/hr
Pressure vs. flow performance	Under 2 bar at flow rate of 300 cm/hr in DI water (20 x 20 cm packed bed, 1.15 compression factor)
Working pH range	3–11
Shipping solution	50% slurry in 20% ethanol
Regeneration	1–2 M NaCl
CIP solutions	6 M guanidine-HCl, 10 mM hydrochloric acid, 0.1 M sodium hydroxide, 1 M acetic acid/20% ethanol
Storage conditions	2–8°C
Chemical stability No significant change in chromatographic performance after storage at RT for 24 hr	10 mM HCl, 6 M guanidine-HCl, 0.1 M arginine (pH 2.8), 0.1 M citrate (pH 2.8), 0.1 M glycine (pH 2.8)
Shelf life	5 years (4–8°C)

Binding Capacity

UNOsphere beads are engineered for high mechanical stability and low backpressures. An increase in binding capacity is achieved with higher bed height (Figure 2) while excellent flow properties are maintained (Figure 3).

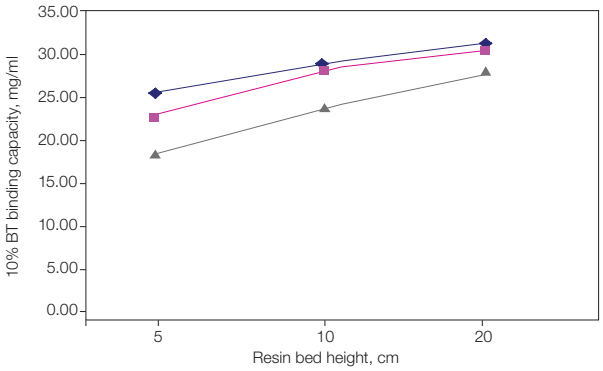


Fig. 2. DBC at various bed heights and flow rates. 150 cm/hr (—◆—); 300 cm/hr (—■—); 400 cm/hr (—▲—).

Pressure/Flow Performance

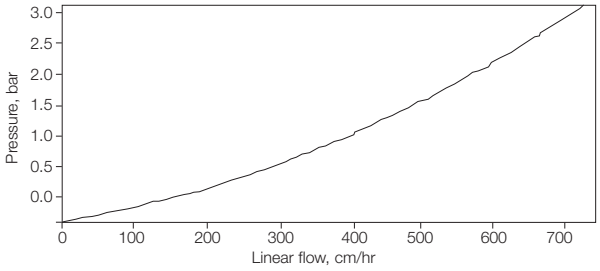


Fig. 3. Flow performance of UNOsphere SUPrA Resin. Column (20 x 20 cm). Compression factor 1.15.

Performance Data

Typically, to effectively elute mAbs from agarose-based Protein A resins, low pH conditions are required. However, low pH can result in higher aggregate content in the recovery pool. Evidence suggests that some mAbs are more susceptible to low pH aggregation or precipitation than others. A unique benefit of the UNOsphere base bead is rapid mass transfer for capture and subsequent release of the target molecule. UNOsphere SUPrA (Protein A ligand) employs this rapid mass transfer to provide less extreme pH conditions with good recovery in low total volumes.

Effects of pH on Aggregate Formation in mAb G Purification

mAb G is sensitive to pH — at pH 7, the mAb is monomeric; at pH 4, low levels of aggregates form and continue to increase with decreasing pH; precipitation occurs at pH 2.8 (Figure 4).

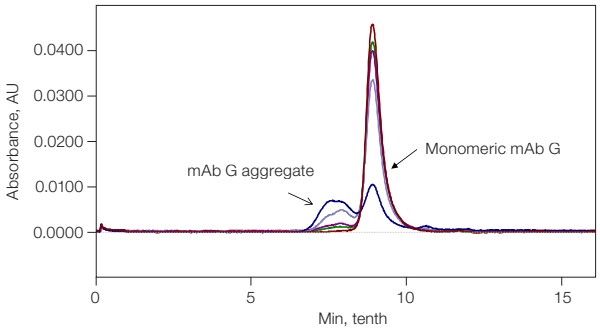


Fig. 4. Effect of pH on mAb G aggregate formation. pH 7.0 (—); pH 4.0 (—); pH 3.5 (—); pH 3.0 (—); pH 2.8 (—).

Recovery of Two mAbs at pH 3.7 Using Selected Protein A Resins

mAb G, a previously purified mAb, and mAb R, a crude CHO feed, were screened on three Protein A resins (Figures 5 and 6). Columns were packed with 1 ml of each resin. At a flow rate of 300 cm/hr, 5.5 mg of each mAb was loaded and washed with 1x PBS followed by elution with 0.1 M glycine at pH 3.7. At pH 3.7, >80% of each mAb was recovered from UNOsphere SUPrA. The mAbs were not eluted efficiently at pH 3.7 for the other resins and were lost in the strip. Total recovery was below 80% even with the large elution volumes.

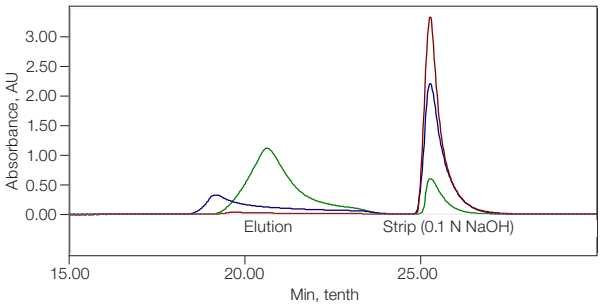


Fig. 5. Elution of mAb G at pH 3.7. UNOsphere SUPrA (—); Resin S (—); Resin R (—).

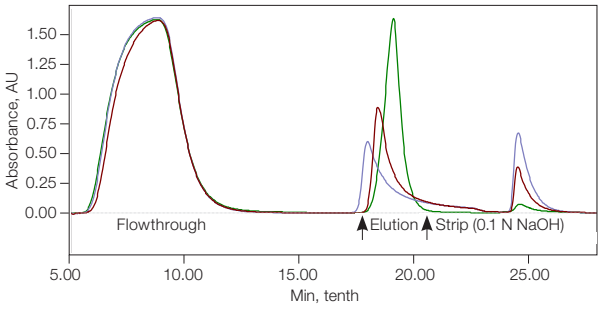


Fig. 6. Elution of mAb R at pH 3.7. UNOsphere SUPrA (—); Resin S (—); Resin R (—).

pH 3.7 was selected due to the sensitivity of mAb G to pH conditions. Since a lower pH is known to improve the performance of the competitor resins, the experiments were repeated at pH 3.5 and the results were compared to those from the pH 3.7 elution (Figure 7). Total percent recovery for both mAb G and mAb R was more consistent with UNOsphere SUPrA than the other resins. Recovery >80% can be achieved in 3–5 CV with UNOsphere SUPrA.

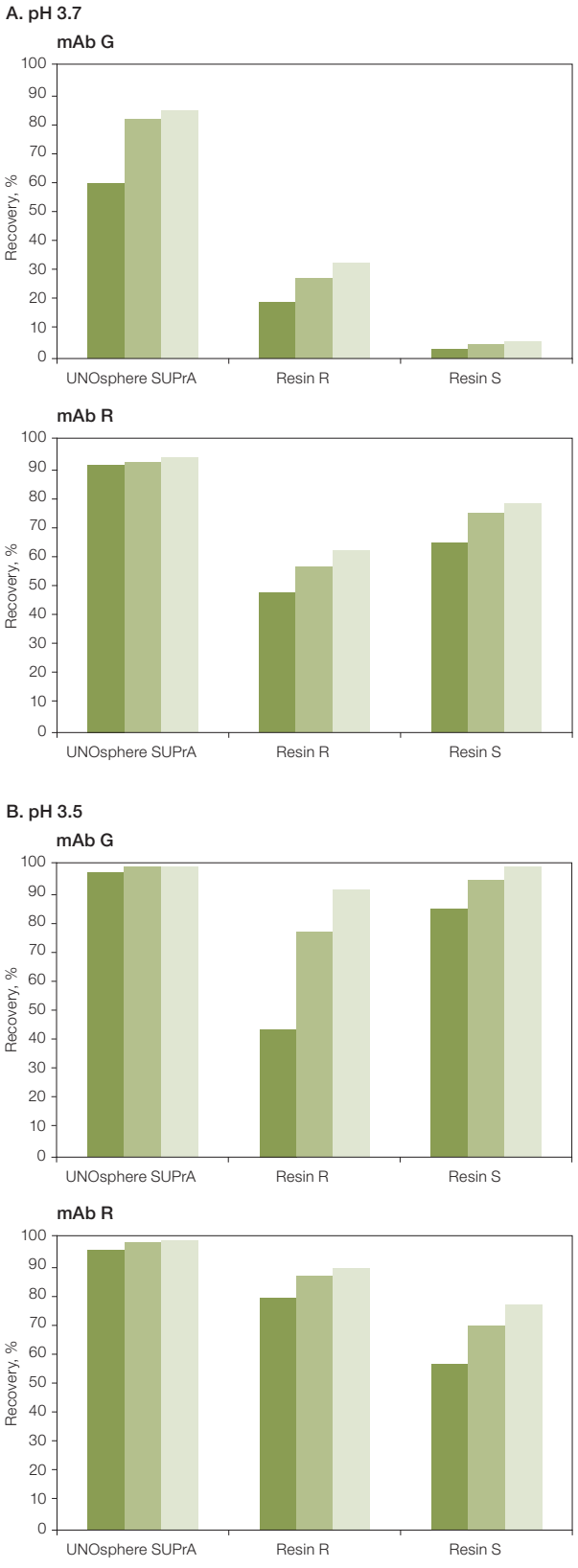
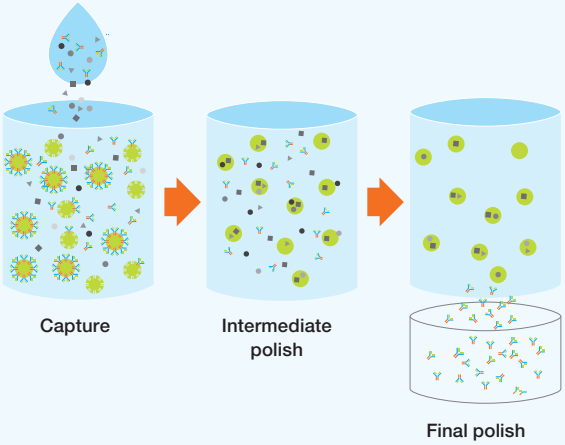


Fig. 7. Comparison of percent recovery using UNOsphere SUPrA and two competitor resins. Percent recovery was evaluated at pH 3.7 (**A**) and pH 3.5 (**B**). 3 CV (■); 5 CV (■); 7 CV (■).

Purification Workflow



mAb1 (chimeric IgG1)

HCP = 175 ng/mg	HCP < 3 ng/mg	HCP < 2 ng/mg
DNA = 18.6 ng/ mg	DNA = 3.5 ng/mg	DNA = 2.5 ng/mg
Protein A = 3 ng/mg	Protein A = 0.8 ng/mg	Protein A < 0.7 ng/mg
		Aggregate <0.03%

Ordering Information

Catalog #	Description
Prepacked Screening Tools	
732-4729	**Foresight™ UNOsphere SUPrA Column**, 1 ml
732-4749	**Foresight UNOsphere SUPrA Column**, 5 ml
732-4834	**Foresight UNOsphere SUPrA RoboColumn Unit**, 200 µl
732-4835	**Foresight UNOsphere SUPrA RoboColumn Unit**, 600 µl

Bulk Resin	
1560218	**UNOsphere SUPrA Affinity Chromatography Media**, 25 ml
1560219	**UNOsphere SUPrA Affinity Chromatography Media**, 100 ml
156-0220	**UNOsphere SUPrA Affinity Chromatography Media**, 500 ml
156-0221	**UNOsphere SUPrA Affinity Chromatography Media**, 5 L
156-0222	**UNOsphere SUPrA Affinity Chromatography Media**, 10 L

Case Studies

The use of UNOsphere SUPrA for capture in a three-step mAb purification process, along with UNOsphere Q and CHT, provides a platform for superior purity and recovery. See page 110 for more information.

Notes

Macro-Prep® Resins

Economical IEX product line for large biomolecule purification, including plasma proteins

Composition and Properties

Polymer	Properties	Derivatives
▪ Polymethacrylate bead matrix, produced as a 50 µm epoxide base polymer ▪ Base polymer is converted in subsequent chemical reactions to various functionalities	▪ Average particle size of 50–70 µm ▪ Macroporous; average pore radius of 60–90 nm ▪ Weak hydrophobic effect ▪ High ligand density	▪ Q and S (strong) ▪ DEAE (weak) ▪ CM (weak) ▪ Methyl and t-butyl HIC

Advantages

Chromatographer	Engineer	Economic buyer
▪ Ion exchangers with weak hydrophobic effect (mixed mode) allow unique selectivity with some target proteins ▪ Strong performance for plasma protein fractionation	▪ Good pressure flow at flow rates up to 600 cm/hr ▪ Favorable economics at scale for cost-sensitive applications ▪ Provides opportunity for customization	▪ Low cost ▪ Long history ▪ Production capacity

INTRODUCTION

Macro-Prep Resins are rigid, macroporous polymethacrylate beads, providing high mechanical strength and low backpressure for bioprocessing applications. The combination of large pores and good flow properties make Macro-Prep useful for a variety of biomolecule purifications. Macro-Prep is produced as a base polymer that is converted to strong and weak IEX derivatives, namely Macro-Prep High Q, Macro-Prep High Q-3HT, Macro-Prep High S, Macro-Prep DEAE, and Macro-Prep CM. The base polymer is also converted into Macro-Prep Methyl and Macro-Prep t-Butyl derivatives for HIC. The average particle size of these products ranges from 50 to 70 µm, and the average pore radius ranges from 60 to 90 nm. Macro-Prep ion exchangers have unique performance capabilities for purification of large biomolecules, including plasma proteins and virus-like particles, due to the large pore structure and mild hydrophobic effect of the polymer backbone.

CATION EXCHANGE

MACRO-PREP RESINS

Macro-Prep® High S Resin

FUNCTIONALITY
- Unique selectivity
- Flow-through purifications
- Cost effective

Macro-Prep High S Resin is a strong cation exchanger containing sulfonate functional groups, ideal for purifying basic and neutral proteins and peptides.

Most polymethacrylate bioprocessing resins including Macro-Prep contain carboxyl groups in the polymer backbone. Depending on the type of resin, pH conditions, and samples, these charges can enhance the mode of interaction. This property can be exploited to provide unique selectivity, increasing yield and purity.

Benefits

- Resolution of complex biological mixtures
- Rigid polymer matrix that allows high flow rates at moderate pressures
- Macroporous beads that enhance access to ionic sites
- Mechanical strength and thermal stability

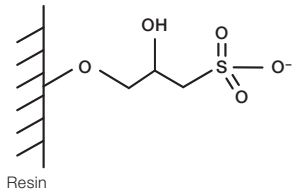


Fig. 1. Macro-Prep High S Resin.

Bead Properties

Property	Description
Type of ion exchanger	Strong cation
Functional group	$-SO_3^-$
Particle size	50 µm
Total ionic capacity	160 ± 40 µeq/ml
Dynamic binding capacity	≥49 mg hIgG/ml
Recommended linear flow rate	50–300 cm/hr
pH stability	1–10*
Shipping solution	20% ethanol + 0.1 M NaCl
Regeneration	1–2 M NaCl/KCl or 70% ethanol
Sanitization	Wash the column with 3–5 CV of 1% acetic acid/1% phosphoric acid, pH 1.5
Storage conditions	20% ethanol or 0.1 N NaOH
Chemical stability	1% SDS, 24 hr 6 M guanidine-HCl, 24 hr
Chemical compatibility	1% SDS, 8 M guanidine-HCl, 1 N HCl, 100% ethanol
Shelf life	5 years

* The use of basic reagents above pH 10 should be evaluated for each application.

Pressure/Flow Performance

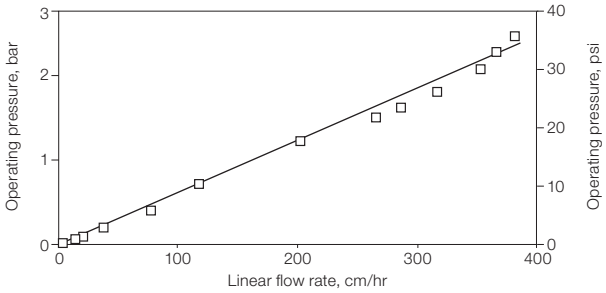


Fig. 2. Operating pressure of 50 µm Macro-Prep Resin in a 14 x 17 cm column.

Performance Data

Macro-Prep High S Resin is an excellent choice for rapid purification. This methacrylate copolymer bead provides high-resolution separations at very high flow rates (Figure 3).

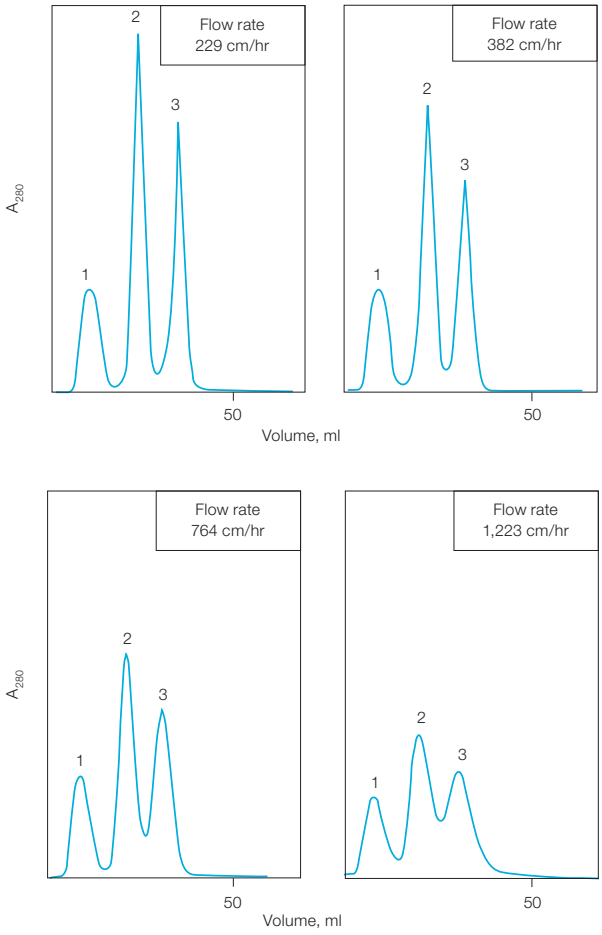
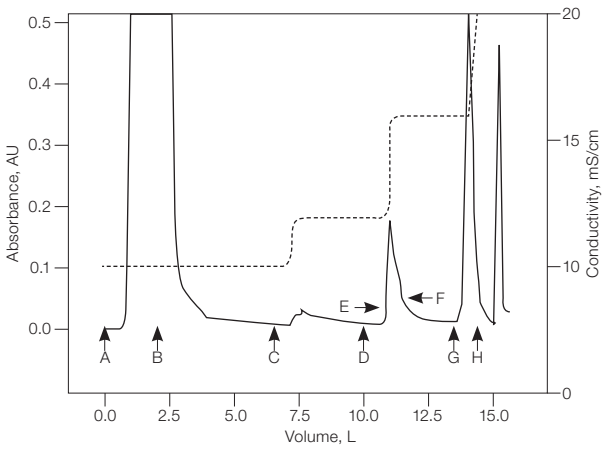


Fig. 3. Effect of flow rate on separation and peak symmetry. A 5 ml sample (1.4 mg/ml) of myoglobin (peak 1), ribonuclease A (peak 2), and cytochrome c (peak 3) was run on a 1 x 13 cm (8.7 ml) column at each of the indicated flow rates. The buffer was 20 mM potassium phosphate, pH 8.0, with a gradient of 0–1 M KCl over 75 ml.

Human monoclonal IgM was purified from bioreactor supernatants in a two-step procedure involving CEX and AEX chromatography. CEX chromatography resulted in 75% purity and a yield of 76% (Figure 4).



Resin:		2,000 ml Macro-Prep High S
Column:		Vantage-S (9 x 50 cm), Amicon
Flow rate:		75 ml/min
Event table:	A–B	Load protein fraction from previous run
	B	50 mM sodium phosphate, pH 7.2
	C	50 mM sodium phosphate, 30 mM NaCl, pH 7.2
	D	50 mM sodium phosphate, 100 mM NaCl, pH 7.2
	E–F	Collect partially purified IgM
	G	50 mM sodium phosphate, 1 M NaCl, pH 7.
	H	1.0 M NaOH

Fig. 4. Purification of IgM using Macro-Prep High S. A_{280} (—); conductivity (– –).

Ordering Information

Catalog #	Description
Prepacked Screening Tools	
7324130	**Bio-Scale™ Mini Macro-Prep High S Cartridges**, 5 x 1 ml
7324132	**Bio-Scale Mini Macro-Prep High S Cartridge**, 1 x 5 ml
7324134	**Bio-Scale Mini Macro-Prep High S Cartridges**, 5 x 5 ml

Bulk Resin	
1580030	**Macro-Prep High S Support**, 25 ml
1560030	**Macro-Prep High S Support**, 100 ml
156-0031	**Macro-Prep High S Support**, 500 ml
156-0032	**Macro-Prep High S Support**, 5 L
156-0033	**Macro-Prep High S Support**, 10 L
156-0034	**Macro-Prep High S Support**, 50 L


CATION EXCHANGE

MACRO-PREP RESINS

Macro-Prep® CM Resin

FUNCTIONALITY
- Unique selectivity
- Weak cation exchanger
- Cost effective

Macro-Prep CM Resin is a weakly acidic CEX resin that meets the demands of analytical, semipreparative, and process-scale applications. This rigid, macroporous hydrophilic resin provides capacity, resolution, and throughput in a mechanically stable form. Macro-Prep CM Resins operate well at low and medium pressures and provide consistent and reproducible results.

Benefits

- Resolution of complex biological mixtures
- Rigid polymer matrix that allows high flow rates at moderate pressures
- Macroporous beads that enhance access to ionic sites
- Mechanical strength and thermal stability

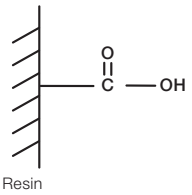


Fig. 1. Macro-Prep CM Resin.

Bead Properties

Property	Description
Type of ion exchanger	Weak cation
Functional group	$-COO^-$
Particle size	50 µm
Total ionic capacity	210 ± 40 µeq/ml
Dynamic binding capacity	≥25 mg hemoglobin/ml
Recommended maximum linear flow rate	50–300 cm/hr
pH stability	1–12
Shipping solution	20% ethanol + 0.1 M NaCl
Regeneration	2–4 CV of a high salt buffer (0.5–2.0 M)
Sanitization	3–5 CV of 1% acetic acid/1% phosphoric acid, pH 1.5
Storage conditions	1% acetic acid in 1% phosphoric acid (pH 1.5) or in 20% (v/v) ethanol solution or in 2% benzyl alcohol
Chemical stability	6 M guanidine-HCl, 24 hr 1% SDS, 24 hr
Shelf life	5 years

Pressure/Flow Performance

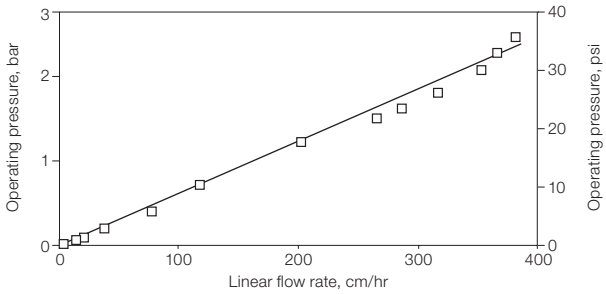


Fig. 2. Operating pressure of 50 µm Macro-Prep in a 14 x 17 cm column.

Ordering Information

Catalog #	Description
Bulk Resin	
1580070	**Macro-Prep CM Support**, 25 ml
1560070	**Macro-Prep CM Support**, 100 ml
156-0071	**Macro-Prep CM Support**, 500 ml
156-0073	**Macro-Prep CM Support**, 10 L

Publications

Application	Reference
Histone purification	Li A et al. (2010). Phosphorylation of histone H2A.X by DNA-dependent protein kinase is not affected by core histone acetylation, but it alters nucleosome stability and histone H1 binding. J Biol Chem 285, 17,778 –17,788.
Clearance of cationic species	Vitali A et al. (1998). Purification and partial characterization of a peroxidase from plant cell cultures of *Cassia didymobotrya* and biotransformation studies. Biochem J 331, 513–519. Porter EM et al. (1998). Isolation of human intestinal defensins from ileal neobladder urine. FEBS Lett 434, 272–276.


ANION EXCHANGE

MACRO-PREP RESINS

Macro-Prep® High Q and Macro-Prep High Q-3HT Resins

FUNCTIONALITY
- Unique selectivity
- Blood fractionation
- Large biomolecule purification
- Cost effective

Macro-Prep High Q and Macro-Prep High Q-3HT Resins are strong anion exchangers containing quaternary amine functional groups, ideal for purifying acidic and neutral proteins and peptides. The two resins differ in their pore size characteristics. These resins are an excellent choice for rapid purification. The Macro-Prep methacrylate copolymer bead provides high-resolution separations at high flow rates.

Most polymethacrylate bioprocessing resins including Macro-Prep contain carboxyl groups in the polymer backbone. Depending on the type of resin, pH conditions, and samples, these charges can contribute to the mode of interaction. This property can be exploited to give unique selectivity, which could increase yield and purity.

Benefits

- Resolution of complex biological mixtures
- Rigid polymer matrix that allows high flow rates at moderate pressures
- Macroporous beads that enhance access to ionic sites
- Mechanical strength and thermal stability

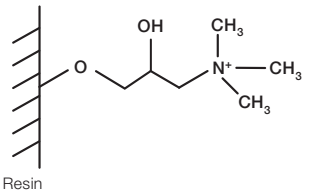


Fig. 1. Macro-Prep High Q and Macro-Prep High Q-3HT Resins.

Bead Properties

Property	Macro-Prep High Q	Macro-Prep High Q-3HT
Type of ion exchanger	Strong anion	Strong anion
Functional group	$-N^+(CH_3)_3$	$-N^+(CH_3)_3$
Particle size	50 µm	50 µm
Total pore volume	0.63–0.72 ml/g	0.75–0.81 ml/g
Total pore area	19–23 m^2/g	24–26 m^2/g
Average pore diameter	1,200–1,450 Å	1,220–1,280 Å
Total ionic capacity	400 ± 75 µeq/ml	400 ± 75 µeq/ml
Dynamic binding capacity	≥37 mg BSA/ml	≥37 mg BSA/ml
Recommended maximum linear flow rate	50–300 cm/hr	50–300 cm/hr
Pressure vs. flow performance	Under 2.5 bar at a flow rate of 300 cm/hr in DI water	Under 2.5 bar at a flow rate of 300 cm/hr in DI water
pH stability	1–10*	1–10*
Shipping solution	20% ethanol + 0.1 M NaCl	20% ethanol + 0.1 M NaCl
Regeneration	2–4 CV of a high salt buffer (0.5–2.0 M)	2–4 CV of a high salt buffer (0.5–2.0 M)
Sanitization	3–5 CV of 1% acetic acid/1% phosphoric acid, pH 1.5	3–5 CV of 1% acetic acid/1% phosphoric acid, pH 1.5
Storage conditions	Short term: 3–5 CV of 1% acetic acid/1% phosphoric acid, pH 1.5 Long term: 20% (v/v) ethanol solution or in 2% benzyl alcohol	Short term: 3–5 CV of 1% acetic acid/1% phosphoric acid, pH 1.5 Long term: 20% (v/v) ethanol solution or in 2% benzyl alcohol
Chemical stability	1% SDS, 24 hr 6 M guanidine-HCl, 24 hr	1% SDS, 24 hr 6 M guanidine-HCl, 24 hr
Shelf life	5 years	5 years

* The use of basic reagents above pH 10 should be evaluated for each application.

Binding Capacity

The dynamic binding capacity of Macro-Prep High Q Resin is unaffected by the linear flow rate (Figure 2).

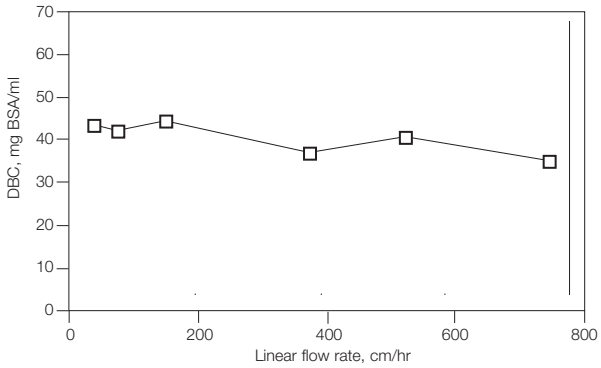


Fig. 2. DBC as a function of linear flow rate. A 100 ml sample (3 mg/ml) of BSA was run on a 1 x 10 cm (8.0 ml) column at the indicated flow rates. The buffer was 50 mM Tris-HCl, pH 8.3. Elution was with 1.0 M NaCl.

Pressure/Flow Performance

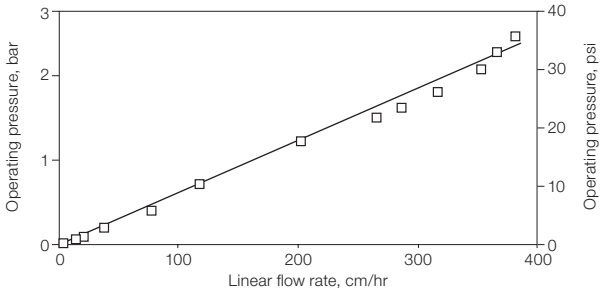


Fig. 3. Operating pressure of 50 µm Macro-Prep Resin in a 14 x 17 cm column.

Performance Data

A panel of resins was evaluated to identify which could be used for endotoxin polishing. Of the 20 resins initially screened for the removal of endotoxin/B-(1,3)-D-glucan from a secreted yeast protein, protein B, six were selected for further evaluation. These resins were equilibrated in PBS, pH 7.4, for endotoxin polishing of 350 ml of protein B, which had been previously purified through a Q Sepharose Fast Flow Gel Column operating in flow-through mode. The samples (5 ml) were passed over each column and the eluates were collected and assayed for the amount of endotoxin in endotoxin units (EU, Figure 4).

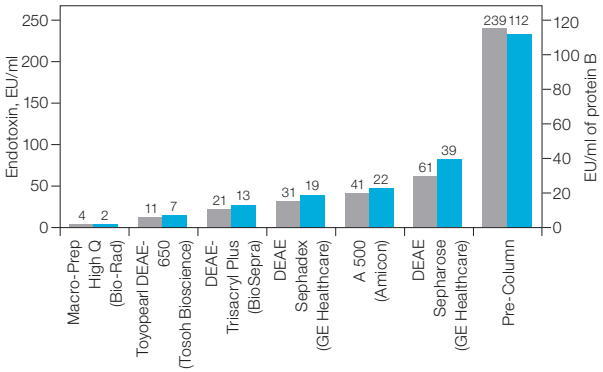


Fig. 4. Results from the evaluation of six different AEX resins for endotoxin removal from protein B. Endotoxin % (■); EU/ml of protein B (■).

Macro-Prep High Q was chosen for the endotoxin polishing step because it resulted in the lowest amount of endotoxin per mg of protein B. The polishing step was performed on a 100 ml column equilibrated with PBS, pH 7.2. The eluate from the previous AEX step was diluted to 3.2 mg/ml and loaded at 5 ml/min. The flow-through fraction, containing protein B, was collected. Figure 5 summarizes the results of the Macro-Prep High Q AEX chromatography, which reduced the endotoxin level from 650 EU/ml to 1.29 EU/ml with a product yield of 84%.

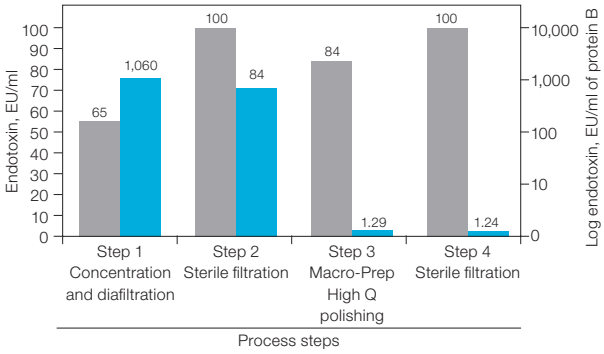


Fig. 5. Summary of results from endotoxin polishing from protein B. Step yield % (■); log endotoxin (■).

Ordering Information

Catalog #	Description
Prepacked Screening Tools	
7324120	**Bio-Scale™ Mini Macro-Prep High Q Cartridges**, 5 x 1 ml
7324122	**Bio-Scale Mini Macro-Prep High Q Cartridge**, 1 x 5 ml
7324124	**Bio-Scale Mini Macro-Prep High Q Cartridges**, 5 x 5 ml

Bulk Resin	
1580040	**Macro-Prep High Q Support**, 25 ml
1560040	**Macro-Prep High Q Support**, 100 ml
156-0041	**Macro-Prep High Q Support**, 500 ml
156-0042	**Macro-Prep High Q Support**, 5 L
156-0043	**Macro-Prep High Q Support**, 10 L
1560043-3HT	**Macro-Prep High Q-3HT Support**, 10 L

MACRO-PREP RESINS

Macro-Prep® DEAE Resin

FUNCTIONALITY
- Unique selectivity
- Weak anion exchanger
- Cost effective

Macro-Prep DEAE derivative is a weak AEX resin developed using a macroporous methacrylate polymer. Macro-Prep DEAE demonstrates excellent flow properties and the ability to maintain its resolving power at flow rates in excess of 1,000 cm/hr. These flow properties, combined with the material's dynamic binding capacity, make Macro-Prep DEAE Resin suitable for processing large sample volumes efficiently.

Macro-Prep DEAE is stable in a wide variety of organic solvents, which makes it possible to thoroughly sanitize and regenerate the material. The combination of elution characteristics and physical stability makes Macro-Prep DEAE a valuable tool for the purification and production of biomolecules.

Most polymethacrylate bioprocessing resins, including Macro-Prep, contain carboxyl groups in the polymer backbone. Depending on the type of resin, pH conditions, and samples, these charges can contribute to the mode of interaction. This property can be exploited to give unique selectivity, which may increase yield and purity.

Benefits

- Resolution of complex biological mixtures
- Rigid polymer matrix that allows high flow rates at moderate pressures
- Macroporous beads that enhance access to ionic sites
- Mechanical strength and thermal stability

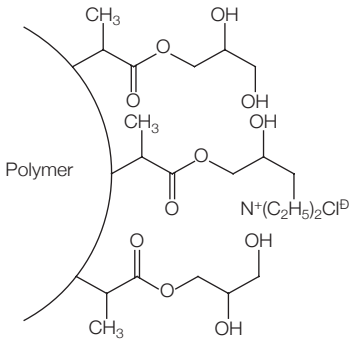


Fig. 1. The Macro-Prep epoxide bead is derivatized with diethylamine to form the weak AEX Macro-Prep DEAE Resin.

Bead Properties

Property	Description
Type of ion exchanger	Weak anion
Functional group	$-N^+(C_2H_5)_2$
Particle size	50 µm
Total ionic capacity	175 ± 75 µeq/ml
Dynamic binding capacity	≥30 mg BSA/ml
Recommended maximum linear flow rate	50–300 cm/hr
pH stability	1–10*
Shipping solution	20% ethanol
Regeneration	2–4 CV of a high salt buffer (0.5–2.0 M)
Sanitization	3–5 CV of 1% acetic acid/1% phosphoric acid, pH 1.5
Storage conditions	1% acetic acid in 1% phosphoric acid (pH 1.5) or in 20% (v/v) ethanol solution or in 2% benzyl alcohol.
Chemical stability	1% SDS, 24 hr 6 M guanidine-HCl, 24 hr
Shelf life	5 years

* The use of basic reagents above pH 10 should be evaluated for each application.

Binding Capacity

Macro-Prep DEAE Resin has high dynamic binding capacity even at high flow rates (Figure 2).

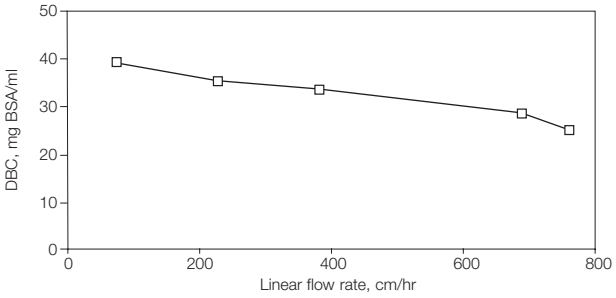


Fig. 2. The effect of flow rate on the DBC of the Macro-Prep DEAE Resin. A sample containing 3 mg/ml BSA was separated on a 1 x 10 cm (3 ml) Macro-Prep DEAE Column. Sample was loaded onto the column in 10 mM Tris-HCl, pH 7.6 (buffer A) and was eluted in 1 M NaCl in buffer A.

Pressure/Flow Performance

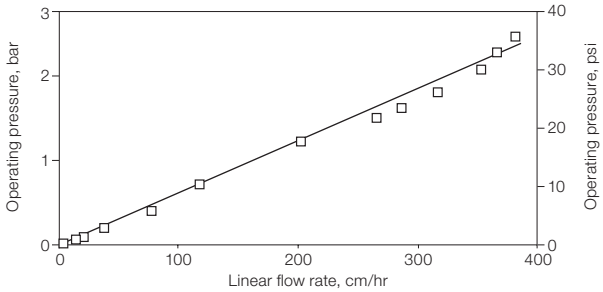


Fig. 3. Operating pressure of 50 µm Macro-Prep Resin in a 14 x 17 cm column.

Performance Data

The particle size distribution of Macro-Prep DEAE Resin is narrow, with a nominal particle size of 50 µm (Figure 4). The porosity of the bead is matched to exact specifications through a precise polymerization process. Regular pores with a narrow size distribution ensure consistent, reliable behavior. The nominal pore size of the bead is 1,000 Å. The resin exhibits consistently high resolution at high flow rates (Figure 5).

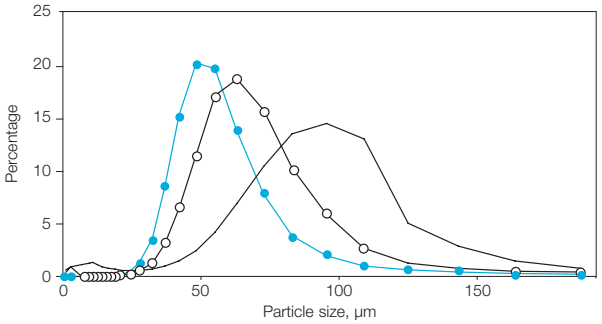


Fig. 4. Particle size distribution of the Macro-Prep DEAE Resin and two other commercially available DEAE resins. Macro-Prep DEAE Resin (—●—); TSK DEAE 650M (—○—); DEAE Sepharose FF (—).

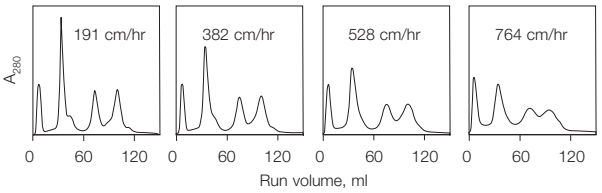


Fig. 5. The effect of flow rate on separation and peak symmetry. A 5 ml sample containing 3.65 mg/ml each myoglobin, conalbumin, BSA, and soybean trypsin inhibitor was loaded onto a column containing 6.56 ml Macro-Prep DEAE Resin. The sample was loaded in 50 mM Tris-HCl, pH 7.6 (buffer A) and eluted in a gradient of 0–1 M NaCl in buffer A as follows: buffer A for 15 ml, 0–0.35 M NaCl for 85 ml, 0.35–0.65 M NaCl for 15 ml, 0.65–1 M NaCl for 15 ml.

In addition, this weak AEX resin shows higher retention times and better separation properties than other commercially available resins (Figure 6). The resin is also able to efficiently handle high sample loads (Figure 7).

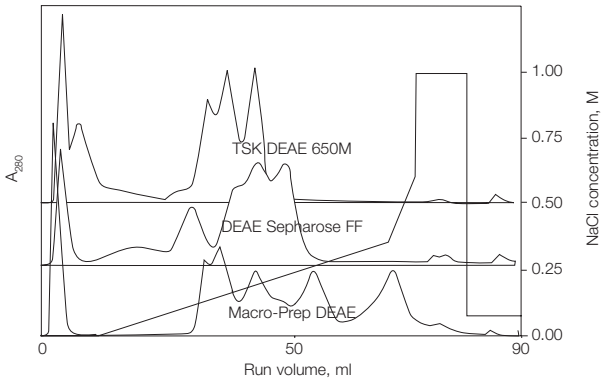


Fig. 6. Sample separation with the Macro-Prep DEAE Resin and two other commercially available DEAE resins. A 100 µl sample containing 9 mg/ml each of myoglobin, conalbumin, ovalbumin, BSA, and soybean trypsin inhibitor was loaded onto a 0.5 cm ID column containing 2 ml of resin. Sample was loaded onto the column in 50 mM Tris-HCl, pH 7.6 (buffer A) and was eluted with 1 M NaCl in buffer A. The flow rate was 153 cm/hr (0.5 ml/min). Scales offset for comparison.

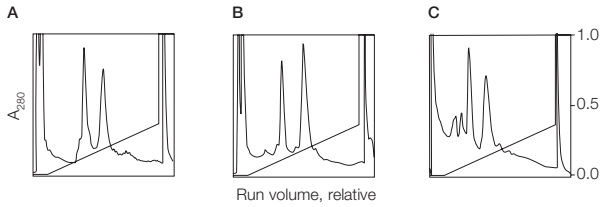


Fig. 7. Scalability of separations on Macro-Prep DEAE Resin. Separation of a yeast enzyme concentrate (Sigma) under various conditions of scale. **A**, a 50 mg sample was separated on a 7.4 ml column (10 mm ID) using a flow rate of 78 cm/hr (1 ml/min) in an analytical flow cell; **B**, a 500 mg sample was separated on a 64 ml column (25 mm ID) using a flow rate of 78 cm/hr (6.4 ml/min) in an analytical flow cell; **C**, a 1 g sample was separated on a 64 ml column (25 mm ID) using a flow rate of 305 cm/hr (25 ml/min) in a preparative flow cell. For all separations, sample was loaded in 50 mM Tris-HCl, pH 8.3, washed with 1 CV of the same buffer, and eluted in a linear gradient of 0–0.35 M NaCl in the same buffer for 22.5 CV.

Ordering Information

Catalog #	Description
Prepacked Screening Tools	
7324140	**Bio-Scale™ Mini Macro-Prep DEAE Cartridges**, 5 x 1 ml
7324142	**Bio-Scale Mini Macro-Prep DEAE Cartridge**, 1 x 5 ml
7324144	**Bio-Scale Mini Macro-Prep DEAE Cartridges**, 5 x 5 ml

Bulk Resin	
1580020	**Macro-Prep DEAE Support**, 25 ml
1560020	**Macro-Prep DEAE Support**, 100 ml
156-0021	**Macro-Prep DEAE Support**, 500 ml
156-0022	**Macro-Prep DEAE Support**, 5 L
156-0023	**Macro-Prep DEAE Support**, 10 L


HYDROPHOBIC INTERACTION

MACRO-PREP RESINS

Macro-Prep® Methyl and Macro-Prep t-Butyl Resins

FUNCTIONALITY
- Strong and weak hydrophobic regions
- Enyzme purification
- Cost effective

Macro-Prep HIC Resins are methacrylate-based 50 µm beads used for protein, polypeptide, enzyme, and nucleic acid purification. They are specifically designed for intermediate purification steps that remove host-cell contaminants from partially purified targets. Due to their rigidity and unique surface chemistry, these hydrophobic chromatography resins are particularly suited for HIC operations requiring high throughput and high recovery of target.

Macro-Prep HIC Resin is available in two functional forms:

- Macro-Prep Methyl HIC Resin — weakly hydrophobic, for purifying compounds that have strongly hydrophobic regions
- Macro-Prep t-Butyl HIC Resin — mildly hydrophobic, for purifying compounds that have few or weakly hydrophobic regions

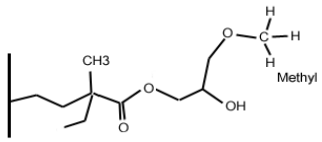
Both HIC resins can be autoclaved and can withstand treatment in acid, chaotropic agents, or detergents while retaining high protein-binding capacities.

Macro-Prep t-Butyl HIC Resin can operate on an interaction mechanism based on both hydrophobicity and charge. The t-butyl groups are mildly hydrophobic. Depending on the pH of the loading and elution buffers, the matrix carboxyl groups can be exploited to ionically bind or repel target molecules or contaminants incombination with hydrophobic interactions. This is an ideal strategy that minimizes the product loss commonly experienced with HIC resin due to denaturation of proteins upon exposure to hydrophobic surfaces.

Benefits

- Hydrophobic interaction selectivity
- Maintenance of biological activity
- Efficient capture of proteins from high-salt conditions

Methyl



t-Butyl

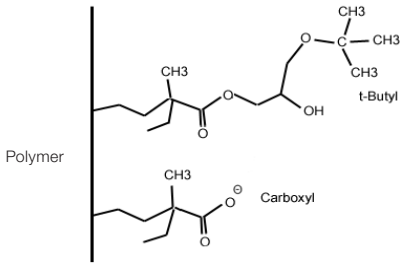


Fig. 1. Macro-Prep HIC Resins: Macro-Prep Methyl contains methyl groups; Macro-Prep t-Butyl contains t-butyl and carboxyl groups.

Bead Properties

Property	Macro-Prep Methyl	Macro-Prep t-Butyl
Hydrophobicity	Weak	Mild
Functional group	Methyl	COO^- and t-butyl
Particle size	50 µm	50 µm
Total ionic capacity	<2 µeq/ml	120 µeq/ml
Dynamic binding capacity	15 mg HSA/ml	25 mg HSA/ml
Recommended maximum linear flow rate	100–600 cm/hr	100–600 cm/hr
Pressure vs. flow performance	<3 bar at 500 cm/hr in DI water	<3 bar at 500 cm/hr in DI water
pH stability	1–10*	1–10*
Shipping solution	20% ethanol	20% ethanol
Regeneration	70% ethanol	In 1–2 M NaCl/KCl or 70% ethanol
Sanitization	5 CV of 6 M guanidine-HCl, 100 cm/hr or 5 CV of 1% acetic acid in 0.12 M phosphoric acid, pH 1.5, 100 cm/hr	5 CV of 6 M guanidine-HCl, 100 cm/hr, 5 CV of 1% acetic acid in 0.12 M phosphoric acid, pH 1.5, 100 cm/hr, or 5 CV 0.15% peracetic acid
Storage conditions	20% ethanol or 1% acetic acid in 0.12 M phosphoric acid, pH 1.5	20% ethanol or 1% acetic acid in 0.12 M phosphoric acid, pH 1.5
Chemical stability	1% SDS, 8 M guanidine-HCl, 1 M HCl, 100% ethanol	1% SDS, 8 M guanidine-HCl, 1 M HCl, 100% ethanol
Shelf life	5 years	5 years

* The use of basic reagents above pH 10 should be evaluated for each application.

Pressure/Flow Performance

The rigidity of Macro-Prep HIC Resins allows high flow rates without bed compression (Figure 2).

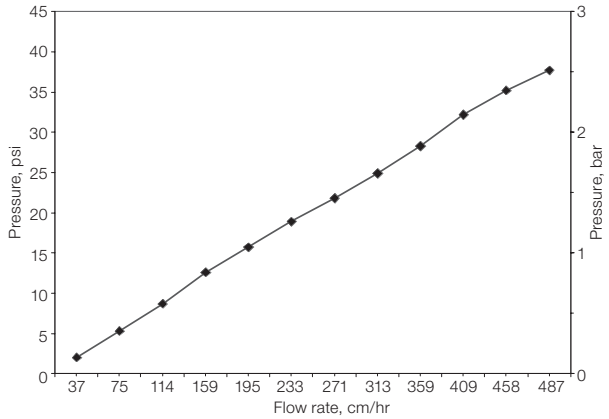


Fig. 2. Pressure vs. flow for Macro-Prep Resins. Resin bed dimensions were 14 cm diameter by 25 cm length.

Performance Data

Macro-Prep Methyl HIC Resin is ideal for purification of proteins with strongly hydrophobic regions. Macro-Prep t-Butyl HIC Resin is ideal for purification of proteins with few or weakly hydrophobic regions. The two different ligands provide alternative selectivities for easier optimization of separation (Figure 3 and Table 1). These methacrylate copolymer beads provide high resolution at very high flow rates.

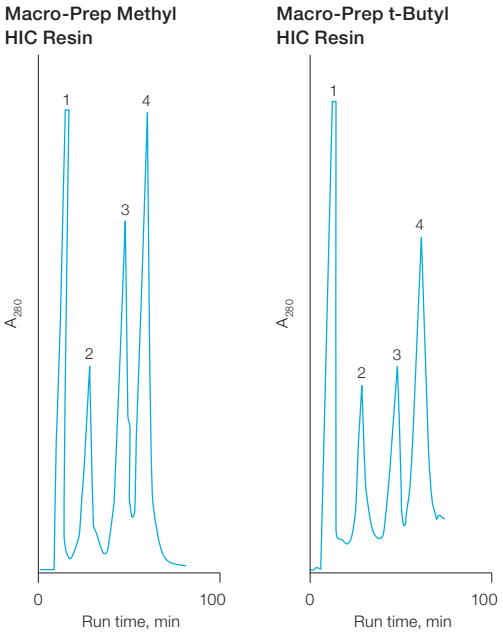


Fig. 3. Sample separation of proteins on Macro-Prep Methyl and t-Butyl Resins. A 50 µl sample (0.6 mg total protein) of cytochrome c (peak 1), ovalbumin (peak 2), α-amylase (peak 3), and ferritin (peak 4) was run on a 1 x 10 cm (4.5 ml) column at a linear flow rate of 38 cm/hr. The sample was eluted with 0.1 M Na_2PO_4, pH 7.0, 1.85 M $(NH_4)_2SO_4$ for 10 min, followed by a gradient from 1.85 to 0 M $(NH_4)_2SO_4$ over 90 min. The retention times for each protein are shown in Table 1 for comparison.

Table 1. Comparison of retention times using Macro-Prep Methyl and t-Butyl ligands.

		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

These methacrylate copolymer beads provide high resolution at very high flow rates. They can be sanitized quickly and efficiently in 0.15% peracetic acid.

Ordering Information

Catalog #	Description
Bulk Resin	
1580080	**Macro-Prep Methyl HIC Support**, 25 ml
1560080	**Macro-Prep Methyl HIC Support**, 100 ml
156-0081	**Macro-Prep Methyl HIC Support**, 500 ml
156-0082	**Macro-Prep Methyl HIC Support**, 5 L
156-0083	**Macro-Prep Methyl HIC Support**, 10 L
1580090	**Macro-Prep t-Butyl HIC Support**, 25 ml
1560090	**Macro-Prep t-Butyl HIC Support**, 100 ml
156-0091	**Macro-Prep t-Butyl HIC Support**, 500 ml
156-0093	**Macro-Prep t-Butyl HIC Support**, 10 L

Ceramic Apatites

The gold standard for removing process- and product-related impurities

Composition and Properties

Polymer	Properties	Derivatives
▪ Nonpolymer-based material ▪ Rigid high purity hydroxyapatite ▪ Pore and particle size controlled by sintering	▪ Two particle sizes — 40 and 80 µm ▪ Superior and broadly applicable selectivity ▪ Multimodal functionality	▪ Hydroxyapatite, Type I and Type II, 40 and 80 µm ▪ Partially fluorinated, Type I, 40 µm ▪ Fluorinated, Type II, 40 µm

Advantages

Chromatographer	Engineer	Economic buyer
▪ Diverse selectivity allowing one-step clearance of product-related contaminants (aggregates, fragments) and host-/process-related contaminants (HCP, endotoxin, virus, and Protein A)	▪ Easy to pack at process scale	▪ Value due to its broad purification performance

INTRODUCTION

Hydroxyapatite is known for its unique selectivity, resolution, and ability to separate proteins shown to be homogeneous by other chromatography techniques. Hydroxypapatite, $(Ca_{10}(PO_4)_6(OH)_2)$, is formed from the chemical combination of calcium and phosphate salts. Unlike most other chromatography media, hydroxyapatite is both the matrix and the ligand. There are two types of binding sites, the positively charged calcium, referred to as C sites, and the negatively charged phosphate, referred to as P sites. Proteins interact via CEX at P sites and via metal affinity at C sites. The IEX interactions are disrupted with increasing conductivity or pH. The metal-chelate interactions are not significantly affected by increased salt concentrations and require a competitive species such as free phosphate for dissociation. As a polishing step, hydroxyapatite separates monomer from dimers and other aggregates and is able to remove HCPs and leached Protein A. DNA elutes after most target proteins due to the strong interactions between the C sites and the DNA phosphate backbone. The interaction with endotoxin is similar, with the phosphate groups in the lipid A moiety and carboxyls in the R polysaccharide contributing to binding.

Ceramic apatites are spherical macroporous beads sintered at high temperatures to modify the nanocrystals into a robust particle that can withstand high flow rates. Ceramic apatites are available as CHT™ Ceramic Hydroxyapatite, MPC™ Ceramic Hydroxyfluoropatite, and CFT™ Ceramic Fluoropatite. CHT, MPC, and CFT retain the unique separation properties of crystalline hydroxyapatite and lot-to-lot control assures reproducibility in large-scale production columns. There are three particles sizes: 20 µm for analytical purposes and 40 and 80 µm for production-scale processes.

Two types of chemically pure ceramic apatites, Type I and Type II, are produced by employing different sintering temperatures. They are distinguished by binding capacity, porosity, and separation profiles. Type I has a higher protein binding capacity whereas Type II has a lower protein binding capacity and a higher resolution of nucleic acids and certain large proteins and viruses. Because Type II has a relatively low affinity to albumin, it can easily be employed for purification of immunoglobulins from plasma.


MIXED MODE

CERAMIC APATITES

CHT™ Ceramic Hydroxyapatite and MPC™ Ceramic Hydroxyfluoroapatite

FUNCTIONALITY
- Highest clearance: aggregate removal
- Dimer removal
- Retroviral clearance
- DNA clearance
- Endotoxin removal

Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, is used in the chromatographic separation of biomolecules. Sets of five calcium doublets (C sites) and pairs of –OH containing phosphate triplets (P sites) are arranged in a repeating geometric pattern. Space-filling models and repeat structure from Raman spectroscopy have also been constructed. Hydroxyapatite has unique separation properties and unparalleled selectivity and resolution. It often separates proteins shown to be homogeneous by electrophoretic and other chromatographic techniques.

CHT Ceramic Hydroxyapatite is a spherical, macroporous form of hydroxyapatite. It has been sintered at high temperatures to modify it from a nanocrystalline to ceramic form. Unlike most other chromatography adsorbents, CHT is both the ligand and the support matrix.

Two types of CHT Ceramic Hydroxyapatite, Type I and Type II, are available for process-scale in two particle sizes — 40 and 80 µm. CHT Type I has a higher protein binding capacity and better capacity for acidic proteins. CHT Type II has a lower protein binding capacity but has better resolution of nucleic acids and certain proteins. The Type II material also has a very low affinity for albumin and is especially suitable for the purification of many species and classes of immunoglobulins.

MPC Ceramic Hydroxyfluoroapatite, $Ca_{10}(PO_4)_6(OH)_{1.5}(F)_{0.5}$, like CHT, is a macroporous ceramic hydroxyapatite with 25% of the hydroxyl groups substituted with fluoride. The incorporation of fluoride creates a more chemically stable form of the matrix.

Benefits
- Unmatched selectivity
- Clearance of impurities and aggregates in a single step
- Rapid and simple column packing

Bead Properties

Property	CHT Type I	CHT Type II	MPC
Function	Mixed-mode, cation (phosphate), and affinity (calcium)	Mixed-mode, cation (phosphate), and affinity (calcium)	Mixed-mode, cation (phosphate), and affinity (calcium)
Functional group	Ca^{2+}, PO_4, OH	Ca^{2+}, PO_4, OH	Ca^{2+}, PO_4, OH, F
Median particle size	20 ± 2, 40 ± 4, 80 ± 8 µm	20 ± 2, 40 ± 4, 80 ± 8 µm	40 ± 4 µm
Dynamic binding capacity	≥25 mg lysozyme/g CHT 25–60 mg IgG/ml CHT*	≥12.5 mg lysozyme/g CHT 15–25 mg IgG/ml CHT*	≥25 mg lysozyme/g MPC 25–50 mg IgG/ml MPC*
Recommended linear flow rate	50–300 cm/hr	50–300 cm/hr	50–300 cm/hr
Maximum operating pressure	100 bar (1,500 psi)	100 bar (1,500 psi)	100 bar (1,500 psi)
Packing density (under ideal conditions)	0.63 g/ml	0.63 g/ml	0.72 g/ml
Compression factor	Incompressible	Incompressible	Incompressible
pH stability	6.5–14	6.5–14	6.5–14
Shipping solution	Dry	Dry	Dry
Regeneration	500 mM sodium phosphate, pH 7; 1 M trisodium phosphate, pH 11–12	500 mM sodium phosphate, pH 7; 1 M trisodium phosphate, pH 11–12	500 mM sodium phosphate, pH 7; 1 M trisodium phosphate, pH 11–12
Sanitization	1–2 N NaOH	1–2 N NaOH	1–2 N NaOH
Autoclavability (bulk)	121°C, 20 min in phosphate, pH 7	121°C, 20 min in phosphate, pH 7	121°C, 20 min in phosphate, pH 7
Storage conditions	0.1 M NaOH + 10 mM sodium phosphate	0.1 M NaOH + 10 mM sodium phosphate	0.1 M NaOH + 10 mM sodium phosphate
Chemical stability	1 M NaOH, 8 M urea, 6 M guanidine-HCl, ethanol	1 M NaOH, 8 M urea, 6 M guanidine-HCl, ethanol	1 M NaOH, 8 M urea, 6 M guanidine-HCl, ethanol
Shelf life	5 years	5 years	5 years

* 40 µm particles, 300 cm/hr, 5 mM sodium phosphate, 25 mM NaCl, pH 6.5
Note: A small amount (up to 5 mM) of sodium phosphate should be added to all unbuffered solutions as a counterion.

Binding Capacity

Hydroxyapatite contains two types of binding sites; positively charged calcium and negatively charged phosphate groups. These sites are distributed throughout the crystal structure of the matrix. Solute species dominantly interact through CEX via the phosphate groups and/or through metal affinity via the calcium atoms.

CEX occurs when protein amino groups interact ionically with the negatively charged phosphates. The amino groups are similarly repelled by the calcium sites. Binding depends on the combined effects of these interactions. These IEX interactions can be disrupted by adding neutral salts, such as sodium chloride, or buffering species, such as phosphate, to the mobile phase. CEX interactions also weaken with increasing pH. Hence, the addition of salt or phosphate or an increase in pH can be used to weaken the interaction. Studies with model proteins have demonstrated that AEX, which might be expected from interactions of negatively charged surface residues with calcium, does not make a significant contribution.

Calcium affinity occurs via interactions with carboxyl clusters and/or phosphoryl groups on proteins or other molecules (for example, nucleic acids); these groups are simultaneously repelled by the negative charge of the CHT phosphate groups. The affinity interaction is between 15 and 60 times stronger than ionic interactions alone and, like classical metal-affinity interactions, is not affected by increasing ionic strength using typical elution ions (for example, chloride). Species binding through calcium affinity may adsorb more strongly as the ionic strength increases due to ionic shielding of the charge repulsion from the CHT phosphate sites. Metal affinity interactions can be dissociated by phosphate in the mobile phase. Figure 1 illustrates CEX metal affinity to carboxyl and phosphoryl groups.

Acidic proteins, such as albumin, bind chiefly by metal-affinity interactions. Sodium chloride at 1.0 M reduces retention time by approximately 10% in the presence of phosphate gradients, indicating a minor contribution by CEX. To elute acidic proteins, phosphate buffers are usually required.

Basic proteins, such as IgG, bind chiefly by CEX interactions and may be selectively eluted with either phosphate or salts.

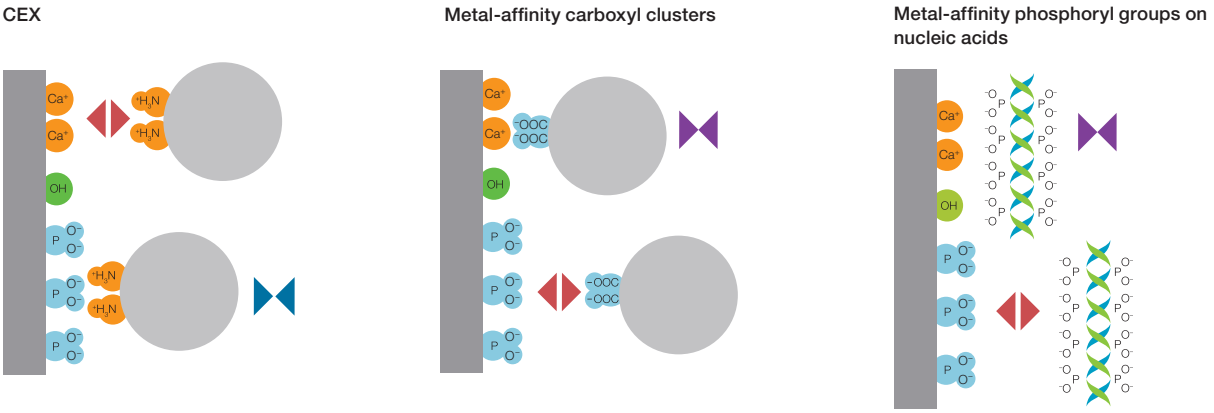


Fig. 1. Schematic representation of CHT binding mechanism. Biomolecule (●); metal affinity (⋈); electrostatic repulsion (⋈); electrostatic attraction (⋈).

Pressure/Flow Performance

CHT and MPC are rigid spheres and typically exhibit low backpressure at high flow rates relative to their average particle sizes (Figure 2; applies to both resins). Figure 3 shows the effect that the different CHT particle sizes have on the separation of proteins.

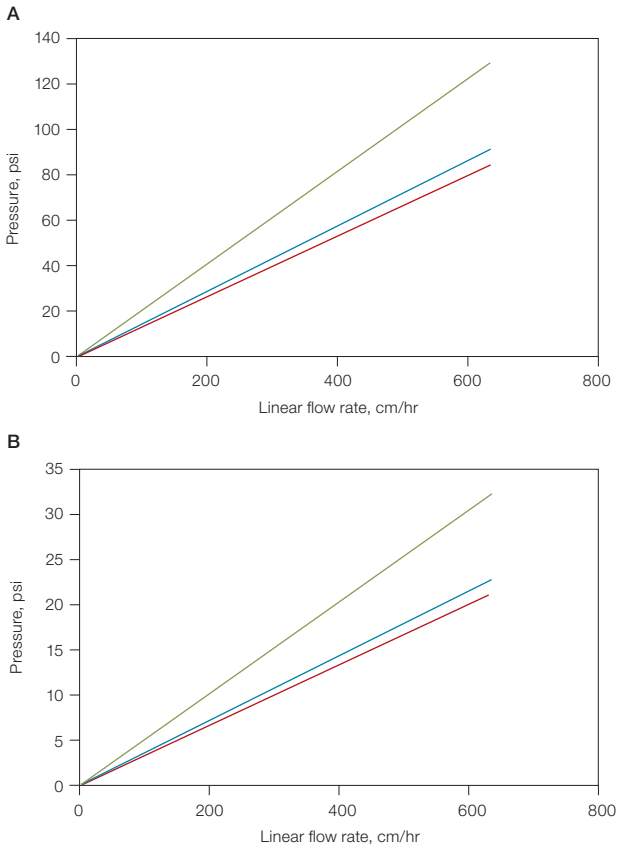


Fig. 2. Estimated pressure for 40 µm CHT (A) and 80 µm CHT (B). Media packed to 20.0 cm bed height vs. 1.0 M NaOH (1.80 CPS), 0.5 M sodium phosphate buffer, pH 6.8 (1.27 CPS), and PBS (1.18 CPS). 1.18 CPS (—); 1.27 CPS (—); 1.80 CPS (—).

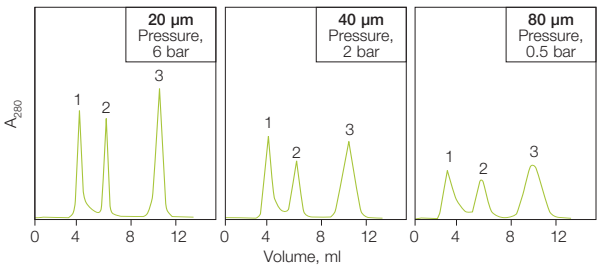


Fig. 3. Effect of particle size on separation of proteins. A 10 µl sample of 10 mg/ml BSA (peak 1), 1.3 mg/ml lysozyme (peak 2), and 5 mg/ml cytochrome c (peak 3) was run on each 4 x 100 mm column packed with the indicated particle size of CHT Ceramic Hydroxyapatite at a flow rate of 478 cm/hr. The elution buffer was a linear gradient of 1–400 mM sodium phosphate, pH 6.8, over 15 min.

Performance Data

CHT and MPC have unique separation properties with unmatched selectivity and resolution, including protein separations (Figure 4) and clearance (Figures 5 and 6; Table 1). Both resins produce similar elution characteristics as shown with five acidic proteins (Figure 7) and nucleotides (Figure 8).

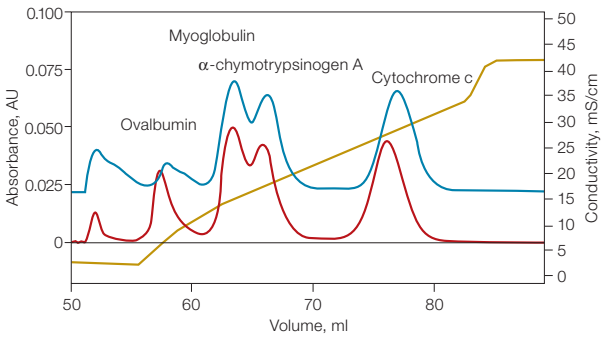


Fig. 4. Separation of protein standards. MPC separates the standard protein mixture similarly to CHT Type I, 40 µm. Sample: 6 mg of ovalbumin, 3 mg of myoglobulin, 3 mg of α-chymotrypsinogen A, and 3 mg of cytochrome c in 1.5 ml 10 mM sodium phosphate (NaPi), pH 6.8. CHT Column: 0.5 x 10.3 cm, packed bed volume 2 ml; MPC Column: 0.5 x 10.4 cm, packed bed volume 2 ml. Protocol: 1.55 ml/min; 400 mM NaPi, pH 6.8, 5 CV; 10 mM NaPi, pH 6.8, 15 CV; load volume 50 µl; linear gradient elution 0 to 75% 400 mM NaPi, pH 6.8, over 15 CV; strip 400 mM NaPi, pH 6.8, 5 CV; sanitization 1 N NaOH, 5 CV. CHT (—); MPC (—); conductivity (—).

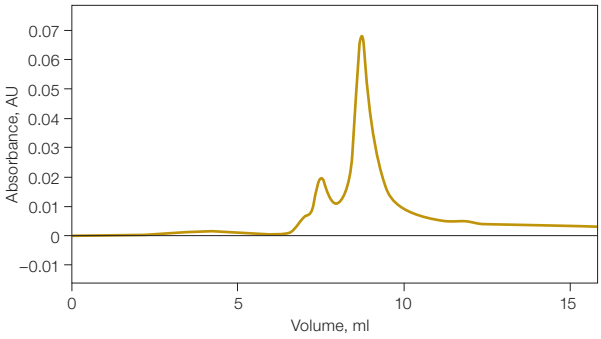


Fig. 5A. mAb-S SEC profile of starting material. SEC profile shows the higher molecular weight impurities (dimers/aggregates) and monomers in the starting material (material applied to the second step (Figure 5B) using CHT or MPC). Sample: 2.9 mg/ml mAb-S in 10 mM NaPi, pH 7. Column: 300 x 7.8 mm, Bio-Sil® SEC 250 HPLC. Protocol: 1 ml/min; equilibration in 1 ml of 0.1 M NaPi, 0.15 M NaCl, 0.02% azide, pH 7; injection volume 0.1 ml; elution in 15 ml of 0.1 M NaPi, 0.15 M NaCl, 0.02% azide, pH 7.

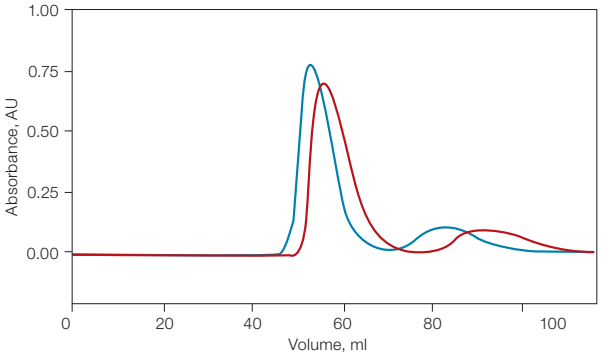


Fig. 5B. mAb-S purification profile. Elution profile shows separation of the monomer from higher molecular weight impurities. Sample: 7.26 mg mAb-S/ml packed bed in 5 ml 10 mM NaPi, pH 7. Column: 0.5 x 10 cm, packed bed volume 2.1 ml of UNOsphere SUPrA™ rProtein A Resin. Protocol: 0.5 ml/min; 10 mM NaPi, pH 7, 5 CV; load volume 5 ml; 10 mM NaPi, pH 7, 5 CV; linear gradient elution 0 to 100% 10 mM NaPi, 1 M NaCl, pH 7, over 40 CV; strip 10 mM NaPi, 1 M NaCl, pH 7, 5 CV; sanitization 1 N NaOH, 5 CV. CHT (—); MPC (—).

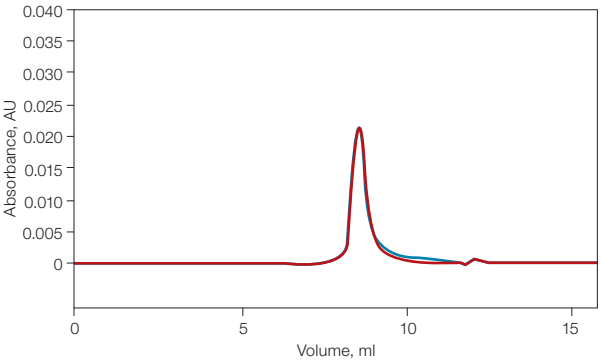


Fig. 5C. SEC profile of pooled monomer fractions. The SEC profile of the pooled fractions confirms aggregate clearance from the monomer. Column: 300 x 7.8 mm, Bio-Sil SEC 250 HPLC. Protocol: 1 ml/min; 100 mM NaPi, 150 mM NaCl, 0.02% azide, pH 7, 1 ml; load volume 0.1 ml; elution 100 mM NaPi, 150 mM NaCl, 0.02% azide, pH 7, 15 ml. CHT (—); MPC (—).

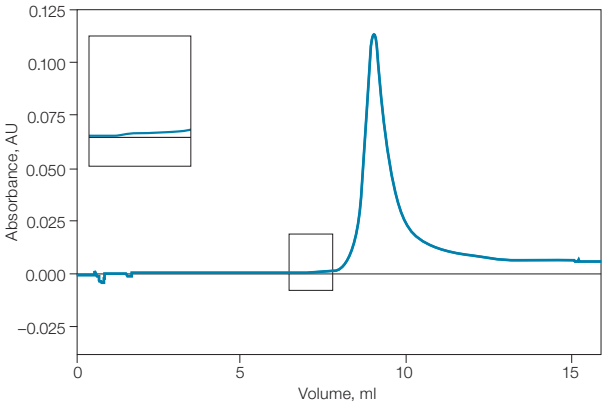


Fig. 6A. mAb-G SEC profile of starting material. SEC profile shows the higher molecular weight impurities (dimers/aggregates) and monomers in the starting material (material applied to the second step (Figure 6B) using CHT or MPC). Sample: 4.41 mg/ml mAb-S in 10 mM NaPi, pH 7. Column: 300 x 7.8 mm, Bio-Sil SEC 250 HPLC. Protocol: 1 ml/min; equilibration in 1 ml of 0.1 M NaPi, 0.15 M NaCl, 0.02% azide, pH 7; injection volume 0.1 ml; elution in 15 ml of 0.1 M NaPi, 0.15 M NaCl, 0.02% azide, pH 7.

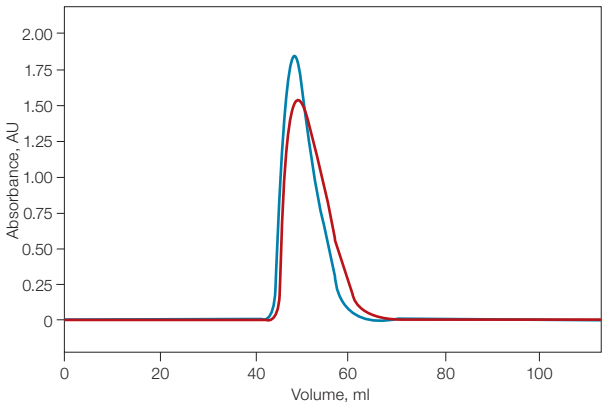


Fig. 6B. mAb-G purification profile. Elution profile shows separation of the monomer from higher molecular weight impurities. Protocol as described in Figure 5B. CHT (—); MPC (—).

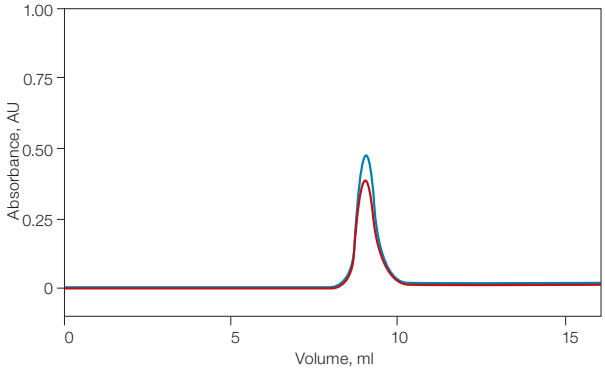


Fig. 6C. SEC profile of pooled monomer fractions. The SEC profile of the pooled fractions confirms aggregate clearance from the monomer. Protocol as described in Figure 5C. CHT (—); MPC (—).

Table 1. Quantified data from all assays. All pooled fractions and mAb starting material purified on MPC and CHT were analyzed for HCPs (HCP ELISA Kit, Cygnus Technologies), dsDNA (Quant-iT PicoGreen dsDNA Assay Kit, Thermo Fisher Scientific Inc.), Protein A leachables (Protein A ELISA Kit, Cygnus Technologies), and aggregate clearance by SEC (Bio-Sil SEC 250, Bio-Rad Laboratories, Inc).

Sample	IgG Concentration, mg/ml	Protein A, ppm	HCP, ppm	DNA, ppm	Dimer/Aggregate, %
MAb-S					
Protein A eluate	2.90	33	24	21	21.62
CHT pool	0.40	<0.6	<2	2.6	<0.03
MPC pool	0.46	<0.5	<2	2.8	<0.03
MAb-G					
Protein A eluate	4.41	17	<2	0.82	4.39
CHT pool	0.91	<0.3	<1	1.4	<0.03
MPC pool	0.77	<0.3	<1	1.4	<0.03

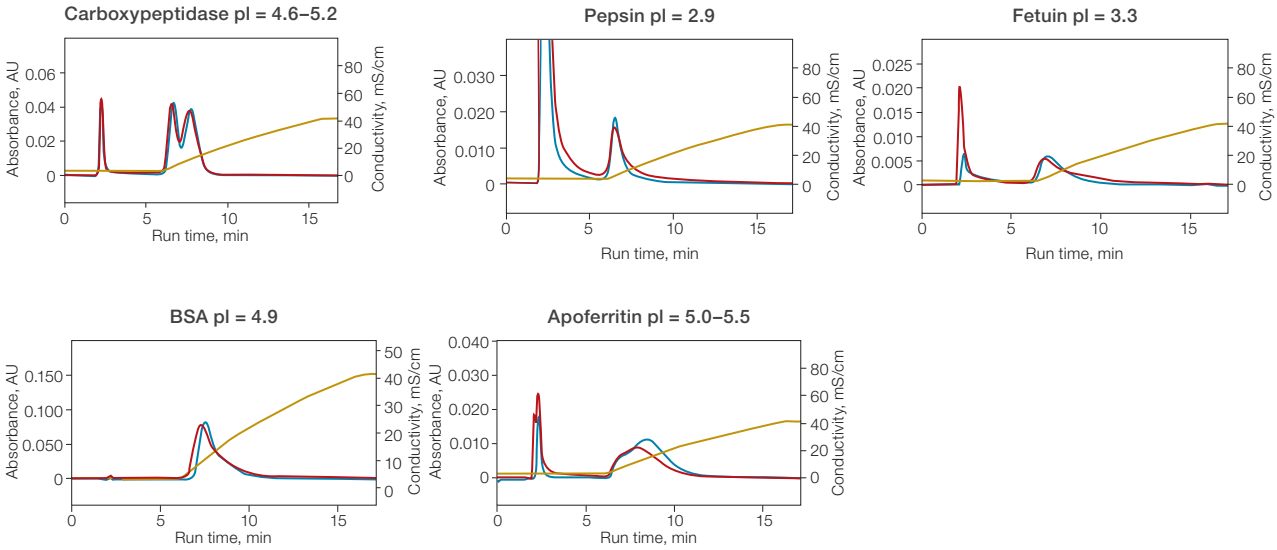


Fig. 7. Elution profiles of five acidic proteins. CHT and MPC produce similar elution characteristics for the five acidic proteins studied. Samples: porcine carboxypeptidase B (CBP), pI 4.6–5.2; pepsin, pI 2.9; fetuin, pI 3.3; BSA, pI 4.9; and apoferritin, pI 5.0–5.5, each prepared to a final concentration of 2–3 mg/ml in water. Column: 0.7 x 2.6 cm, packed bed volume 1 ml, executed using a BioLogic DuoFlow™ System and BioLogic Duoflow Software. Protocol: 300 cm/hr; load volume 100 µl; linear gradient elution 0 to 100% 0.02 M HEPES, 0.01 M NaPi, pH 6.5, to 0.02 M HEPES, 0.4 M NaPi, pH 6.5, over 20 CV. CHT (—); MPC (—); conductivity (—).

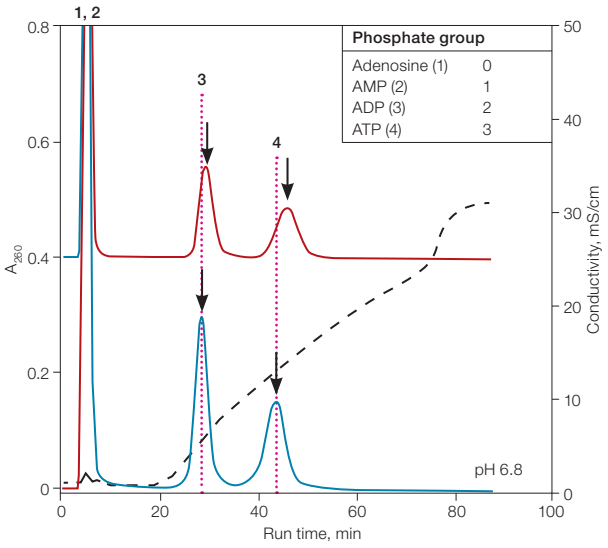
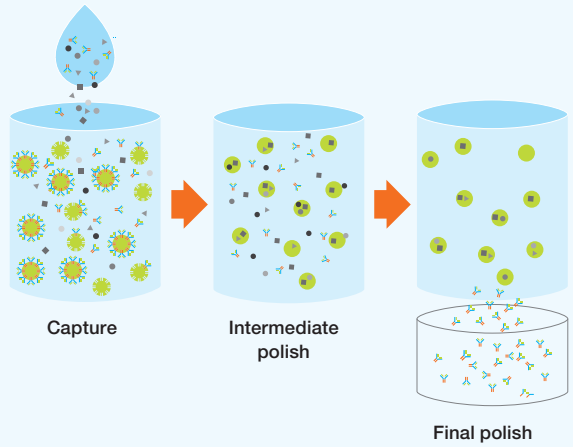


Fig. 8. Nucleotide separation profile. Elution profile shows separation of ADP and ATP. Sample: 1 mg each of adenosine, AMP, ADP, and ATP mixture dissolved in 1 ml of 10 mM NaPi, pH 6.8, filtered with 0.22 µm membrane. Column: 0.4 x 10 cm. Protocol: 1 ml/min; load volume 0.03 ml; wash 10 mM NaPi, pH 6.8, 2 CV; linear gradient elution 0 to 75% 400 mM NaPi, pH 6.8, over 12 CV; strip 400 mM NaPi, pH 6.8, 4 CV. CHT (—); MPC (—); conductivity (- -).

Purification Workflow



Ordering Information

Catalog #	Description
Prepacked Screening Tools	
Foresight™ Columns	
732-4737	**Foresight MPC Type I Column**, 40 µm, 1 ml
732-4757	**Foresight MPC Type I Column**, 40 µm, 5 ml
732-4735	**Foresight CHT Type I Column**, 40 µm, 1 ml
732-4755	**Foresight CHT Type I Column**, 40 µm, 5 ml
732-4736	**Foresight CHT Type II Column**, 40 µm, 1 ml
732-4756	**Foresight CHT Type II Column**, 40 µm, 5 ml
Foresight Plates*	
732-4785	**Foresight MPC Type I Plates**, 40 µm, 20 µl
732-4716	**Foresight CHT Type I Plates**, 40 µm, 20 µl
732-4718	**Foresight CHT Type II Plates**, 40 µm, 20 µl
Foresight RoboColumn Units**	
732-4822	**Foresight CHT Type I RoboColumn Unit**, 40 µm, 200 µl
732-4823	**Foresight CHT Type I RoboColumn Unit**, 40 µm, 600 µl
732-4825	**Foresight CHT Type II RoboColumn Unit**, 40 µm, 200 µl
732-4826	**Foresight CHT Type II RoboColumn Unit**, 40 µm, 600 µl
732-4828	**Foresight MPC Type I RoboColumn Unit**, 40 µm, 200 µl
732-4829	**Foresight MPC Type I RoboColumn Unit**, 40 µm, 600 µl

* 2 x 96-well plates
** Package size: one row of eight columns

Catalog #	Description
Bulk Resin	
CHT Ceramic Hydroxyapatite, Type I	
1584000	**CHT Ceramic Hydroxyapatite**, 40 µm, Type I, 10 g
1570040	**CHT Ceramic Hydroxyapatite**, 40 µm, Type I, 100 g
157-0041	**CHT Ceramic Hydroxyapatite**, 40 µm, Type I, 1 kg
157-0045	**CHT Ceramic Hydroxyapatite**, 40 µm, Type I, 5 kg
1588000	**CHT Ceramic Hydroxyapatite**, 80 µm, Type I, 10 g
1570080	**CHT Ceramic Hydroxyapatite**, 80 µm, Type I, 100 g
157-0081	**CHT Ceramic Hydroxyapatite**, 80 µm, Type I, 1 kg
157-0085	**CHT Ceramic Hydroxyapatite**, 80 µm, Type I, 5 kg
CHT Ceramic Hydroxyapatite, Type II	
1584200	**CHT Ceramic Hydroxyapatite**, 40 µm, Type II, 10 g
1574000	**CHT Ceramic Hydroxyapatite**, 40 µm, Type II, 100 g
157-4100	**CHT Ceramic Hydroxyapatite**, 40 µm, Type II, 1 kg
157-4500	**CHT Ceramic Hydroxyapatite**, 40 µm, Type II, 5 kg
1588200	**CHT Ceramic Hydroxyapatite**, 80 µm, Type II, 10 g
1578000	**CHT Ceramic Hydroxyapatite**, 80 µm, Type II, 100 g
157-8100	**CHT Ceramic Hydroxyapatite**, 80 µm, Type II, 1 kg
157-8500	**CHT Ceramic Hydroxyapatite**, 80 µm, Type II, 5 kg
MPC Ceramic Hydroxyfluoroapatite, Type I	
1580200	**MPC Ceramic Hydroxyfluoroapatite**, 40 µm, Type I, 10 g
1570200	**MPC Ceramic Hydroxyfluoroapatite**, 40 µm, Type I, 100 g
157-0201	**MPC Ceramic Hydroxyfluoroapatite**, 40 µm, Type I, 1 kg
157-0205	**MPC Ceramic Hydroxyfluoroapatite**, 40 µm, Type I, 5 kg

Case Studies

The use of CHT for final polishing in a three-step mAb purification process, along with UNOsphere SUPrA and UNOsphere Q, provides a platform for superior purity and recovery. See page 110 for more information.

CHT and MPC can be used for the purification of a wide variety of mammalian viruses at process scale. The method development is simple, easily scalable, and results in a concentrated preparation of highly active virus. See page 132 for more information.

Commercially available mixed-mode media were compared under optimized bind and elute conditions for the purification of mAb S. CHT provided the best monomer recovery in the smallest elution volume at 99.5% purity. See page 146 for more information.


MIXED MODE

CERAMIC APATITES

CFT™ Ceramic Fluoroapatite

FUNCTIONALITY
- Highest clearance: aggregate removal
- Dimer removal
- Retroviral clearance
- DNA clearance
- Endotoxin removal

CFT Ceramic Fluoroapatite, $Ca_{10}(PO_4)_6F_2$, is a rigid, spherical macroporous media used in the purification of biologically active compounds. CFT is apatite-based media and is a composite of fluoroapatite and hydroxyapatite prepared by chemically converting hydroxyapatite nanocrystals to fluoroapatite with a fluorine reagent. CFT is a multimodal chromatographic media that interacts with biomolecules through CEX via phosphate groups, through metal affinity via calcium atoms, or both. Unlike most other chromatography media, fluoroapatite is both the matrix and the ligand, providing multiple modes of interaction.

CFT Ceramic Fluoroapatite possesses separation characteristics similar to CHT™ Ceramic Hydroxyapatite (see page 58 for more information). However, with CFT, purifications can be performed at lower pH. In addition, CFT can be used under stringent chromatography conditions to separate acidic proteins with minimal compromise to the solubility or lifespan of the apatite.

The unique selectivity of CFT Ceramic Fluoroapatite is attributed to its multiple interactions with biomolecules. Amino groups are attracted to phosphate (P) sites but repelled by calcium (C) sites; the situation is reversed for carboxyl groups. This is similar to CHT and MPC™ Ceramic Hydroxyfluoroapatite.

Benefits

- Acidic protein separation for applications requiring pH as low as 5.0
- Sintered at high temperature for a heavy-duty durable support
- Rigid particles for fast flow rates
- Inorganic calcium phosphate backbone for distinct selectivities

Bead Properties

Property	Description
Function	Mixed-mode, cation (phosphate), and affinity (calcium)
Functional group	Ca^{2+}, PO_4^{3-}, F^-
Median particle size	40 ± 4 µm
Dynamic binding capacity	14–21 mg lysozyme/ml 33 mg IgG/ml
Recommended linear flow rate	50–300 cm/hr
Maximum operating pressure	100 bar (1,500 psi)
Packing density	0.86 g/ml
Compression factor	Incompressible
pH stability	Operating pH: 5–14 Storage pH: 11–14
Shipping solution	Dry
Regeneration	Normal conditions: 400 mM sodium phosphate, pH 7.4; Difficult conditions: 400–1,000 mM sodium phosphate, pH 11–12
Sanitization	1–2 M NaOH or KOH; autoclavability (121°C, 20 min)
Storage conditions	20% ethanol or 0.1 N NaOH
Chemical stability	2 M NaOH, 6 M guanidine-HCl, 8 M urea, 0.1 M sodium acetate, pH 5.7
Shelf life	5 years

Note: A small amount (up to 5 mM) of sodium phosphate should be added to all unbuffered solutions as a counterion.

Binding Capacity

CFT Type II is available in a 40 µm particle size and is sintered at high temperatures to produce physically and chemically stable bioprocess resins. It maintains its highest IgG binding capacity (34–37 mg/ml) at pH 5.0–6.0 (Figure 1) and over a range of flow rates (Figure 2).

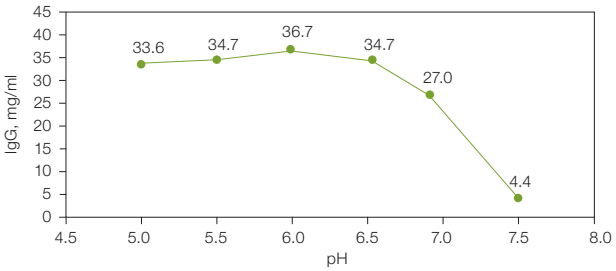


Fig. 1. IgG binding vs. pH using CFT Ceramic Fluoroapatite.

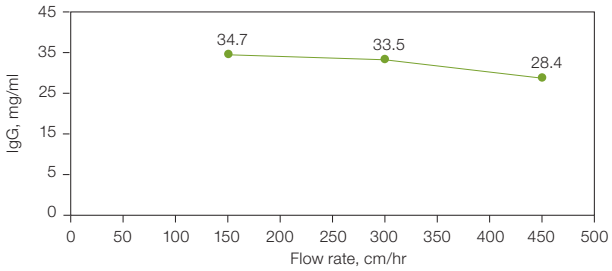


Fig. 2. IgG binding vs. flow rate using CFT Ceramic Fluoroapatite.

Performance Data

Antibodies have a wide variety of uses in analytical and diagnostic applications. They are also a major biotherapeutic agent for treatment of human diseases. Protein A and Protein G affinity separations, IEX, and gel filtration chromatography are techniques commonly used for these types of separations. The following data describe the purification of a chimeric IgG mAb using a two-step purification platform consisting of a UNOsphere SUPrA™ Resin capture followed by a CHT Ceramic Hydroxyapatite or CFT Ceramic Fluoroapatite polishing step. The results demonstrate that both CHT and CFT deliver highly purified chimeric IgG by removing leached Protein A, HCPs, DNA, and mAb aggregates.

The analytical SEC chromatogram of the starting tissue culture supernatant is shown in Figure 3. Evaluation of the UNOsphere SUPrA Resin affinity purification pool in Figure 4 indicates a high degree of IgG purity, with approximately 0.8% dimers and 0.4% aggregates.

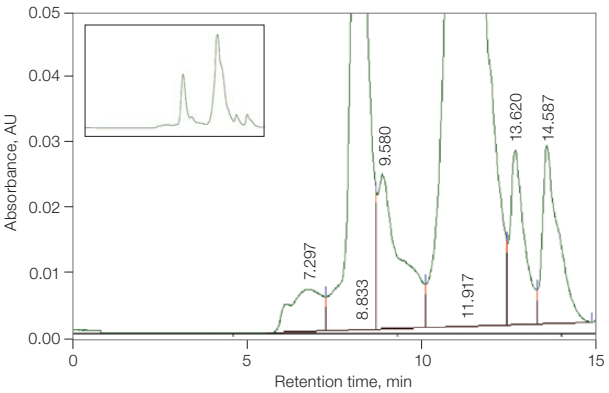


Fig. 3. Evaluation of starting tissue culture supernatant. A sample of chimeric IgG mAb supernatant derived from CHO cells was analyzed by HPLC-SEC to evaluate initial levels of contamination. The peak at 8.8 min represents the chimeric IgG. Inset shows zoomed out view of chromatogram.

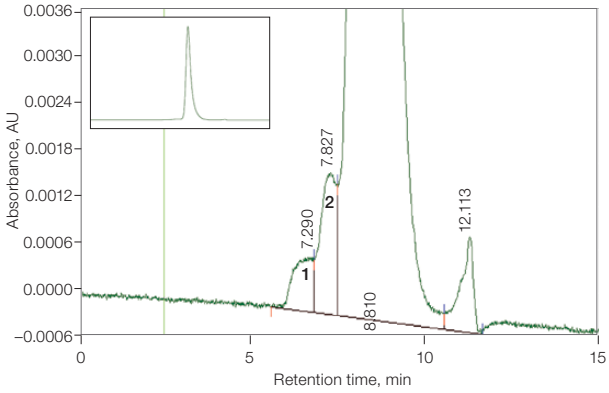


Fig. 4. Capture of chimeric IgG by UNOsphere SUPrA Affinity Resin. Elution fractions from the chromatographic capture step were analyzed by HPLC-SEC to evaluate levels of contamination. Peak 1 and peak 2 correspond to dimer contaminants and aggregate contaminants, respectively. Inset shows zoomed out view of chromatogram.

The antibody peak on the CHT Column eluted later (~69 mS/cm) than did the antibody peak on the CFT Column (~54 mS/cm), indicating that the mAb has a higher affinity for CHT than for CFT (Figure 5).

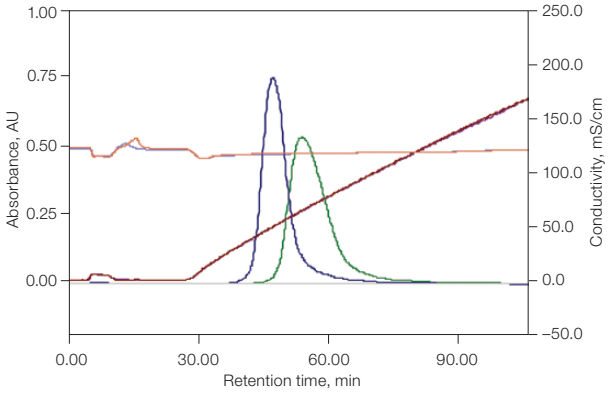


Fig. 5. Comparison of CFT and CHT Media in the final polishing of chimeric IgG. The blue and green traces correspond to chimeric IgG undergoing final polishing by CFT chromatography and by CHT chromatography, respectively. The salt gradient profiles of the two chromatographic purifications are superimposed.

Analysis of the CHT and CFT pools by HPLC-SEC showed no evidence (<0.03%) of dimers or aggregates (Figure 6). This represents higher purity than that of the supplied chimeric IgG standard (Table 1).

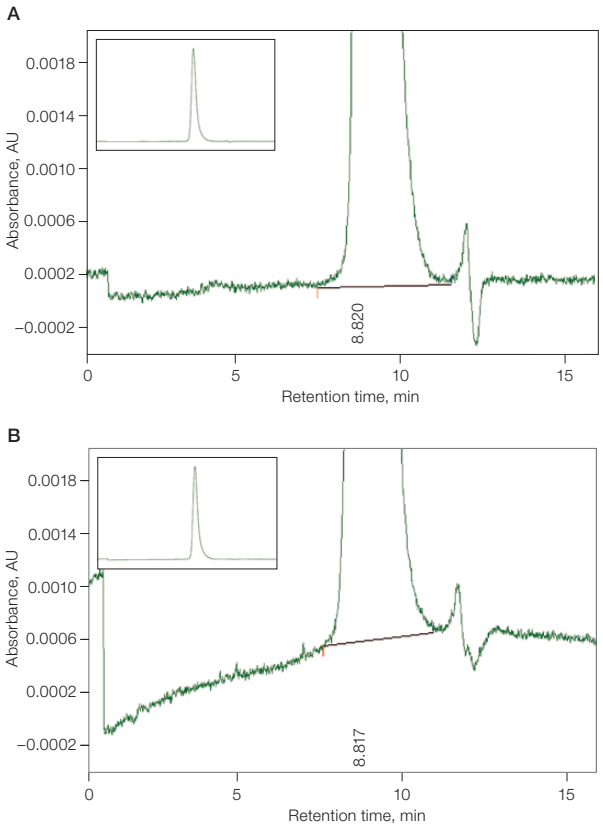


Fig. 6. Removal of dimer and aggregate impurities by CHT and CFT Media chromatography. Elution fractions from the final polishing step using CHT (**A**) or CFT (**B**) were analyzed by HPLC-SEC to evaluate levels of contamination. Dimer and aggregate levels of less than 0.03% were detected. Insets show zoomed out view of chromatograms.

Table 1. Chimeric IgG recovery and impurity clearance.

	IgG, mg/ml	Protein A, ppm	HCP, ng/mg	HCP reduction, log	DNA, ppm	DNA reduction, log	Dimer/ Aggregate, %
Tissue culture	1	NA	5.94×10^4	—	7.58×10^3	—	2.48
UNOsphere SUPrA Resin pool	2.03	3.17×10^1	1.18	>3	9.57	>2	1.24
CHT pool	0.52	<0.96	2.5	>4	5.90×10^1	>4	<0.03
CFT pool	0.66	<0.75	1.7	>4	1.18	>3	<0.03
Chimeric IgG standard	10	10.98	139.3	—	0.87	—	2.47

The comparison performance data for CHT Ceramic Hydroxyapatite and CFT Ceramic Fluoroapatite demonstrate that the media are suitable as a second polishing step in a chimeric IgG mAb purification process.

Ordering Information

Catalog #	Description
Prepacked Screening Tools	
7324405	**Bio-Scale™ Mini CFT Type II Cartridges**, 1 x 5 ml
7324406	**Bio-Scale Mini CFT Type II Cartridges**, 5 x 5 ml
Bulk Resin	
1585200	**CFT Ceramic Fluoroapatite**, Type II, 40 µm, 10 g
1575000	**CFT Ceramic Fluoroapatite**, Type II, 40 µm, 100 g
157-5100	**CFT Ceramic Fluoroapatite**, Type II, 40 µm, 1 kg
157-5500	**CFT Ceramic Fluoroapatite**, Type II, 40 µm, 5 kg

Case Studies

CFT can be used for the purification of a wide variety of mammalian viruses at process scale. The method development is simple, easily scalable, and results in a concentrated preparation of highly active virus. See page 132 for more information.

Specialty Beads

Highly purified and sized to provide consistent, reproducible results across industrial applications in a variety of particle sizes and compositions

Composition and Properties

Polymer	Properties	Derivatives
▪ AG® Resin: styrene divinylbenzene ▪ Chelex® Resin: styrene divinylbenzene ▪ Bio-Beads™ Resin: styrene and polystyrene divinylbenzene	▪ Available in 2%, 4%, 8%, and 12% crosslinkages and various ionic forms ▪ Available in 1% crosslinkage, sodium form ▪ Bead size 300–1,180 µm, adsorption of organics <2,000 daltons ▪ Bead size 40–80 µm; exclusion limits range: 400—14,000 daltons; 1% crosslinked, used by gravity flow; 3% crosslinked, used up to 5 ml/min, 20 bar; 8% and 12% crosslinked, used up to 33 bar	▪ AG 1, AG 2, AG 50W, AG 501 ▪ Chelex 100 ▪ Bio-Beads SM-2 ▪ Bio-Beads S-X

Advantages

Chromatographer	Engineer	Economic buyer
▪ AG: separation of low molecular weight molecules (inorganic ions, organic acids, nucleic acids, carbohydrates) ▪ AG 501: used for buffer purification ▪ Chelex: ultrapurification of buffers and ionic reagents ▪ Bio-Beads SM-2: removal of detergents (Triton X-100), removal of organic substances ▪ Bio-Beads S-X: fractionation of organic polymers, hydrophobic substances (pesticides, unsaturated lipids) ▪ Biotechnology grade available for select resins	▪ Favorable economies of scale ▪ Provides opportunity for customization	▪ Low cost ▪ Historical resins referenced in common industrial applications

INTRODUCTION

AG Analytical Grade Resins are a microporous copolymer of styrene and divinylbenzene. They are produced with various degrees of permeability and different functional groups and are available as both strong and weak cation and anion exchangers as well as mixed-bed ion exchangers. They are extensively purified to remove both organic and inorganic impurities. The resins are sized to give consistent narrow wet mesh ranges, which provide high resolution and excellent reproducibility for laboratory- to process-scale applications.

They are available in several ionic forms and can be converted from one ionic form to another. They are primarily employed for the separation of low molecular weight molecules, such as inorganic ions, and small molecules like organic acids, nucleic acids, and carbohydrates.

Chelex Resins are styrene divinylbenzene copolymers containing iminodiacetate ions. These act as chelating groups in binding polyvalent metal ions with high selectivity and are used to remove metal ions from samples and buffers.

Bio-Beads SM-2 Resins are nonpolar microporous polymeric beads with high surface area for adsorbing organics with molecular weights less than 2,000 daltons. They are often used in aqueous solution with solvents including alcohols, petroleum ether, diethyl ether, and hexane and in solvent mixtures without expansion or contraction of beads.

Bio-Beads S-X Resins are neutral, porous styrene divinylbenzene beads for SEC of lipophilic polymers and other solutes requiring organic eluants for solubility. Exclusion limits range from 400 to 14,000 daltons.

CATION EXCHANGE

SPECIALTY BEADS

AG® 50 Resin

FUNCTIONALITY

- Ultrapurification of buffers and ionic reagents
- Sample preparation
- Metal separations
- Weak acid separations
- Peptide separations
- Amino acid separations
- Nucleotide separations

AG 50 Strong Acid CEX Resins are composed of sulfonic acid functional groups attached to a styrene divinylbenzene copolymer lattice. The amount of resin crosslinking determines the bead pore size. A resin with lower crosslinkage has a more open structure, permeable to higher molecular weight substances than a highly crosslinked resin. It also has lower physical resistance to shrinking and swelling, so that it absorbs more water and swells to a larger wet diameter than a highly crosslinked resin of equivalent dry diameter. Typical applications of 2% crosslinked AG 50W-X2 Resin and 4% crosslinked AG 50W-X4 Resin include separation or concentration of peptides, nucleotides, and amino acids. Resins with high percentage crosslinkage, such as 8% crosslinked AG 50W-X8 Resin, 12% crosslinked AG-50W-X12 Resin, and 16% crosslinked AG 50W-X16 Resin, are used for the separation of small peptides and amino acids, removal of cations, and metal separations. Industrial applications also include water softening, nitrate removal, purification of sugar compounds, pharmaceutical, and fermentation applications. Table 1 shows approximate molecular weight exclusions for each percent crosslinkage.

Benefits

- Extensively purified to remove impurities
- Sized to provide high resolution and reproducibility
- Strong basic cation exchanger
- Converts to several ionic forms easily

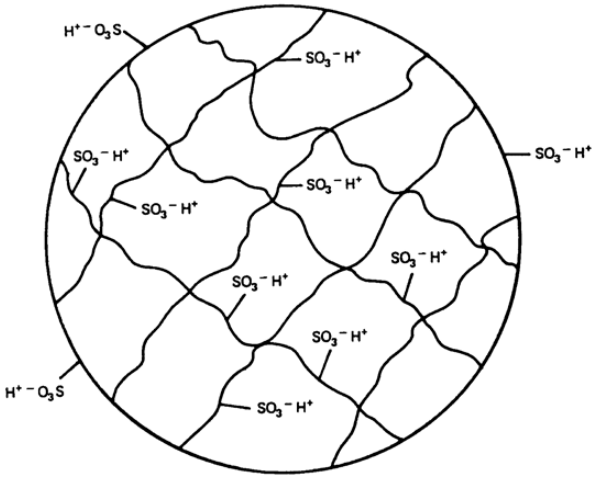


Fig. 1. AG 50 Resin functional group.

Table 1. Approximate molecular weight exclusion limits for ion exchange resins in water.

Percent Crosslinking	Approximate MW Exclusion Limit for Globular Molecules
2%	2,700
4%	1,400
8%	1,000
10%	800
12%	400

When using IEX resins, the counterions on the resin are replaced by sample ions that have the same charge. In applications involving a CEX resin, such as AG 50 Resin, neutral molecules and anions do not interact with the resin. AG 50 Resin is available with H^+, Na^+, or NH^{3+} counterions. A resin can be converted from one ionic form to another. Usually the resin is used in an ionic form with a lower selectivity for the functional group than the sample ions to be exchanged. The sample ions are then exchanged when introduced and can be eluted by introducing an ion with higher affinity for the resin or a high concentration of an ion with equivalent or lower affinity. Table 2 shows the relative selectivity of various counterions.

In general, the lower the selectivity of the counterion the more readily it exchanges for another ion of like charge. The order of selectivity can also be used to estimate effectiveness for different ions as eluants, with the most highly selective being the most efficient. Finally, the order of selectivity can be used to estimate the difficulty of converting the resin from one form to another. Conversion from a highly selected to a less highly selected form requires an excess of the new ion.

Table 2. Relative selectivity of various counterions.

Counterion	Relative Selectivity for AG 50W-X8 Resin	Counterion	Relative Selectivity for AG 50W-X8 Resin
H^+	1.0	Fe^{2+}	2.55
Li^+	0.85	Zn^{2+}	2.7
Na^+	1.5	Co^{2+}	2.8
NH_4^+	1.95	Cu^{2+}	2.9
K^+	2.5	Cd^{2+}	2.95
Rb^+	2.6	Ni^{2+}	3.0
Cs^+	2.7	Ca^{2+}	3.9
Cu^+	5.3	Sr^{2+}	4.95
Ag^+	7.6	Hg^{2+}	7.2
Mn^{2+}	2.35	Pb^{2+}	7.5
Mg^{2+}	2.5	Ba^{2+}	8.7

Bead Properties

Property	Description
Type of ion exchanger	Strong cation
Functional group	SO_3^-
Minimum wet capacity	2% crosslinkage: 0.6 meq/ml 4% crosslinkage: 1.1 meq/ml 8% crosslinkage: 1.7 meq/ml 12% crosslinkage: 2.1 meq/ml Macroporous (MP): 1.5 meq/ml
Nominal density	2% crosslinkage: 0.7 g/ml 4% crosslinkage: 0.8 g/ml 8% crosslinkage: 0.8 g/ml 12% crosslinkage: 0.85 g/ml MP: 0.8 g/ml
Recommended linear flow rate based on application	Removing trace ions: 5–10 cm/min Separations with very few components: 1–3 cm/min Separations of multicomponent samples: 0.3–1.0 cm/min Using high-resolution resins with small particle size: 0.1–0.2 cm/min
Pressure vs. flow performance	See Table 3
pH stability	0–14
Shipping solution	Wet mesh
Regeneration	Equilibrate with 5 CV 3 M HCl followed by 8 CV distilled water
Sanitization	Resins are stable in acid, base, and organic solvents and may be autoclaved
Storage conditions	Ambient temperatures
Chemical stability	Resins are stable in acid, base, and organic solvents and may be autoclaved (except the OH form)
Shelf life	Resins are stable for at least two years when stored in the original unopened container at ambient temperatures and protected from ultraviolet light

Pressure/Flow Performance

The flow rate in a chromatography column increases with increasing particle size. However, the attainable resolution increases with decreasing particle size and narrower size distribution ranges. Particle size is given either in mesh size or micron size. The larger the mesh size number, the smaller the particle size. Large mesh material (20–50 and 50–100 mesh) is used primarily for preparative applications and batch operations where the resin and sample are slurried together. Medium mesh resin (100–200 mesh) may be used in batch as well as column applications. Medium mesh is an ideal general purpose particle size for use in analytical and preparative scale column chromatography. Fine mesh material (200–400 and –400 mesh) is used for high-resolution analytical separations.

Table 3. Wet mesh and equivalent micron diameters.

Wet Mesh (U.S. Standard)											
16	20	40	50	80	100	140	200	270	325	400	
Micron Diameter (1 µm = 0.001 mm)											
1,180	850	425	300	180	150	106	75	53	45	38	

Publications

Cation Exchangers for Sample Preparation

Application	Resin	Reference
Cation removal from monosaccharides	AG 50W-X8	Ochiai M (1980). J Chromatogr 194, 224.
Removal of cations from sulfate	AG 50W-X8	Hoffer EM et al. (1979). Atmospheric Environment 13, 303.
Metal removal	AG 50W-X8	Siemer DD (1980). Anal Chem 52, 1874.
Cyclic nucleotide extraction	AG 50W-X8	Schwartz JP et al. (1973). J Biol Chem 248, 2699; Kuo W et al. (1973). J Biol Chem 248, 2705.
Concentration of vitamin B-6	AG 50W-X8	Tryfiates GP and Sattsangi S (1982). J Chromatogr 227, 181.
Concentration of amino acids	AG 50W-X8	Ford CW (1984). J Sci Food Agric 35, 881.
Removal of contaminants from I^{125}	AG 50W-X8	Auf'mkolk M (1986). J Biol Chem 261, 11,623.
Concentration of chloramphenicol	AG 50W-X8	Schwartz DP and McDonough FE (1984). J Assoc Off Anal Chem 67, 583.
Removal of ethidium bromide from plasmids	AG 50W-X8	Rodriguez RL and Tait RC (1983). In: Recombinant DNA Techniques: An Introduction (Boston: Addison-Wesley Publishing Company), pp. 153–154.
Concentration of isomers of trans-2, 3-cis-3,4-dihydroxyl-L-proline	AG 50W-X8	Linblad WJ and Diegelmann RF (1984). J Chromatogr 315, 447.
Isolation of neutral and cationic metabolites	AG 50W-X8, AG 1-X8	Terry RC and Simon MJ (1982). Chromatogr 232, 261.
Deionization of N-nitro-sodiethanolamine	AG 50W-X8	Wigfield YY and Lanouette M (1985). Assoc Off Anal Chem 68, 1142.
Deionization of carbohydrates	AG 50W-X8, AG 1-X8	Cullen MP et al. (1985). J Chromatogr 337, 29.
Concentration of nucleotide fragments	AG 50W-X8	Kapian BB et al. (1978). Biochemistry 17, 5516.
Concentration of 3-methyl-L-histidine	AG 50W-X8	Robert JC and Serog P (1984). Clin Chim Acta 142, 161.
Separation of adeno-syl-L-methionine from amino-cyclopropane carboxylic acid	AG 50W-X4	Miura GA and Chiang DK (1985). Anal Biochem 147, 217.
N-acetyl-L-[^{35}S] Met purification	AG 50W	Martin DJ and Rubenstein PA (1987). J Biol Chem 262, 6350.
Nitrite determination in meat	AG 50W-X12	Kordorouba V and Pelletier M (1988). Mitt Geb Lebensmitteiunters Hyg 79, 90.
Glycopeptide and oligosaccharide purification	AG 50W-X2	Nishikawa Y et al. (1988). J Biol Chem 263, 8270.
Aldehyde and ketone separation	AG 50W-X2	Rendina AR and Cleland WW (1981). Anal Biochem 117, 213.
Diethyl acetal purification	AG 50W-X8	Cho YK et al. (1988). Biochemistry 27, 3320.
Ammonia determination in plasma	AG 50W-X8	Forman DT (1964). Clinical Chem 10, 497.
Metal removal	AG 50W-X8	Graf E (1983). J Agric Food Chem 31, 851.
Boron cleanup	AG 50W-X8	Gregorie D (1987). Anal Chem 59, 2479.
Amino acid concentration	AG 50W-X8	Stabler SP et al. (1987). Anal Biochem 162, 185.
Peptide-Ch 6-S purification	AG 50W-X8	Takagaki K et al. (1988). J Biol Chem 263, 7000.
Free calcium removal from bound Ca-G-actin	AG SCW-X8	Zimmerle CT and Frieden C (1988). Biochemistry 27, 7759.
Aspartic acid purification	AG 50	MacKenzie SL and Tenaschuk J (1985). J Chromatogr 322, 228.

Cation Exchangers for Sample Preparation, cont.

Application	Resin	Reference
Glutamic acid K	AG 50	MacKenzie SL and Tenaschuk J (1985). J Chromatogr 322, 228.
Tetrabutylammonium fluoride removal	AG 50W-X2	Chou S-H et al. (1989). Biochemistry, 28, 2422.
Peptide cleanup	AG 50W-X2	Schiffmann E et al. (1975). J Immunol 114, 1831.
L-tryptophan purification	AG 50W-X2	Yoshida R et al. (1988). J Immunol 141, 2819.
Taurine cleanup	AG 50W-X8	Stephan ZF et al. (1987). J Biol Chem, 262, 6069.
Glyphosate quantitation	AG 50W-X8	Thompson et al. (1989). JAOAC 72, 355.
cAMP purification	AG 50W-X8	Nemecek GM et al. (1979). J Biol Chem 254, 598.

Metal Separations on Cation Exchangers

Metals	Recommended Resin	Eluant and Eluted Ions	Reference
Bi, Cd, Fe, Cu, Mn, Ni	AG 50W-X8	Bi-50% acetone, 0.1 M HCl; Cd-70% acetone, 0.2 M HCl; Fe - 80% acetone, 0.5 M HCl; Cu-90% acetone, 0.5 M HCl; Mn-92% acetone, 1 M HCl; Ni-aqueous 3 M HCl	Fritz JS and Fettig TA (1962). Anal Chem 34, 1562.
V, U, Sc, Y	AG 50W-X8	V - 0.25 M H_2SO_4; U - 0.5 M H_2SO_4; Sc - 1 M H_2SO_4; Y 4 N HCl	Strelow FWE et al. (1965). Anal Chem 37, 106.
Be, Ba, Sr	AG 50W X8	Be, Ba-9 M $HCl0_4$; Sr-5 M $HN0_3$	Nelson F et al. (1984). J Chromatogr 13, 503.
K, Ti, Sc	AG 50W X8	K-9 M $HClO_4$; Ti-9 M HCl; Sc-4 M HCl, 0.1 M HF	Nelson F et al. (1964). J Chromatogr 13, 504.

Application	Resin	Reference
Metal separation (Pm, Y, Eu, Co, Fe, Am, Cm, Nd)	AG 50W-X12	Jerome SM (1988). The Science of the Total Environment 70, 275.
^{111}In separated from cyclotron target	AG 50W-X4	Van der Walt TN et al. (1985). Int J Appl Radiat Isot 36, 501.
Cobalt separation	AG 50W-X4	Victor AH (1983). S Afr J Chem 36, 76.
Trace metal separation	AG 50W-X4	Van der Walt TN and Strelow FWE (1983). Anal Chem 55, 212.
Thorium detection	AG 50W-X4	Victor AH and Strelow FWE (1982). Anal Chim Acta 138, 285.
Trace element separation from manganese	AG 50W-X8	Faisca AMMM et al. (1988). Anal Chim Acta, 215, 317.
Rare earth element separation	AG 50W-X8	Juras SJ et al. (1987). Chem Geol 64, 143.
Metal separation (Th, Fr, UO)	AG 50W-X8	Paunescu N (1986). J Radioanal Nucl Chem 104, 205.

Application	Resin	Reference
Platinum and palladium determination	AG 50W-X8	Brown RJ and Biggs WR (1984). Anal Chem 56, 646.
Chromium thiocyanate hydrate analysis	AG 50W-X8	Collins CH and Lancas FM (1983). Radiochem Radioanal Lett 56, 117.
Copper determination	AG 50W-X8	Victor AH (1983). Geostand Newsl 7, 227.
Rare earth element determination	AG 50W-X8	Savoyant L et al. (1984). Geostand Newsl 8, 159.
Lead separations	AG MP-50	Strelow FWE (1985). Anal Chem 57, 2268.
Copper detection	AG 50	Lazaro F et al. (1988). Anal Chim Acta 214, 217.
Iron detection in wine	AG 50W-X8	Ajlec R and Stupar J (1989). Analyst 114, 137.
Rare earth metal separation	AG 50W-X8	Hiramatsu K and Yamada T (1988). Jpn Kokai Tokkyo Koho.

Cation Exchange Resins in Enzymatic Assays

Application	Resin	Reference
Separation of acetylglutamate from glutamate	AG 50W-X8	Alonso E and Rubio V (1985). Anal Biochem 146, 252.
Adenylate cyclase assay	AG 50W-X4	Marcus R and Orner F (1977). Endocrinol 101, 1570.
Adenylate cyclase assay	AG 50W-X4	Salomon Y et al. (1974). Anal Biochem 58, 541.
GABA aminotransferase assay	AG 50W-X8	Silverman RS and George C (1988). Biochemistry 27, 3285.
cAMP separation from ATP	AG 50W-X4	Kowluru RA et al. (1989). Biochemistry 28, 2220.

Ordering Information

Catalog #	Ionic Form	Mesh Size	Nominal Capacity, meq/ml	Diameter, µm	Nominal Density, g/ml	Package Size, kg
Bulk Resins						
AG 50W-X2 Resin						
142-1231	Hydrogen	50–100	0.6	300–1,180	0.70	0.5
142-1232	Hydrogen	50–100	0.6	300–1,180	0.70	10
142-1241	Hydrogen	100–200	0.6	106–300	0.70	0.5
142-1242	Hydrogen	100–200	0.6	106–300	0.70	10
142-1251	Hydrogen	200–400	0.6	75–180	0.70	0.5
AG 50W-X2 Resin, Biotechnology Grade						
1435241	Hydrogen	100–200	0.6	106–300	0.70	0.1
AG 50W-X4 Resin						
142-1331	Hydrogen	50–100	1.1	180–425	0.80	0.5
142-1341	Hydrogen	100–200	1.1	106–250	0.80	0.5
142-1351	Hydrogen	200–400	1.1	75–150	0.80	0.5
AG 50W-X4 Resin, Biotechnology Grade						
1435341	Hydrogen	200–400	1.1	75–150	0.80	0.1
AG 50W-X8 Resin						
142-1421	Hydrogen	20–50	1.7	300–1,180	0.80	0.5
142-1423	Hydrogen	20–50	1.7	300–1,180	0.80	10
142-1431	Hydrogen	50–100	1.7	180–425	0.80	0.5
142-1432	Hydrogen	50–100	1.7	180–425	0.80	10
142-1441	Hydrogen	100–200	1.7	106–250	0.80	0.5
142-1442	Hydrogen	100–200	1.7	106–250	0.80	10
142-1451	Hydrogen	200–400	1.7	63–150	0.80	0.5
142-1452	Hydrogen	200–400	1.7	63–150	0.80	10
AG 50W-X8 Resin, Molecular Biology Grade						
1431451	Sodium	200–400	1.7	63–150	0.80	0.1
AG 50W-X8 Resin, Biotechnology Grade						
1435441	Hydrogen	100–200	1.7	106–250	0.80	0.1
1435451	Hydrogen	200–400	1.7	63–150	0.80	0.1
AG 50W-X12 Resin						
142-1254	Hydrogen	200–400	2.1	53–106	0.85	1
142-1641	Hydrogen	100–200	2.1	106–250	0.85	0.5
142-1651	Hydrogen	200–400	2.1	53–106	0.85	0.5
AG MP-1M Resin						
141-1831	Chloride	50–100	1.0	150–300	0.70	0.5
141-1841	Chloride	100–200	1.0	75–150	0.70	0.5
141-1842	Chloride	100–200	1.0	75–150	0.70	10
141-1851	Chloride	200–400	1.0	38–75	0.70	0.5
141-1852	Chloride	200–400	1.0	38–75	0.70	10
141-1853	Nitrate	200–400	1.0	38–75	0.70	0.5
AG MP-50 Resin						
143-0841	Hydrogen	100–200	1.5	75–150	0.80	0.5

SPECIALTY BEADS

AG® 1 and AG 2 Resins

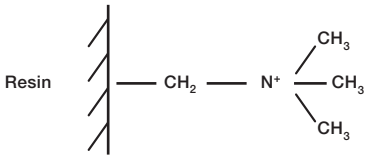
FUNCTIONALITY
- Ultrapurification of buffers and ionic reagents
- Metal separations
- Peptide separations
- Amino acid separations
- Nucletoide separations
- Nitrate removal
- Purification of sugar compounds
- Fermentation applications

AG 1 and AG 2 Resins are strongly basic anion exchangers with quaternary ammonium functional groups attached to the styrene divinylbenzene copolymer lattice. The amount of resin crosslinkage determines the bead pore size. A resin with a lower percentage of crosslinkage has a more open structure permeable to higher molecular weight substances than a highly crosslinked resin. It also has a lower physical resistance to shrinking and swelling, so that it absorbs more water and swells to a larger wet diameter than a highly crosslinked resin of equivalent dry diameter. They are capable of exchanging anions of acidic, basic, and neutral salts, and ampholytes on the basic side of their pI. These strong AEX resins are typically used for sample preparation, enzyme assays, metal separations, and peptide, protein, and nucleic acid separations.

Benefits

- Strong basic anion exchanger
- Extensively purified to remove impurities
- Sized to provide high resolution and reproducibility
- Converts to several ionic forms easily

AG 1



AG 2

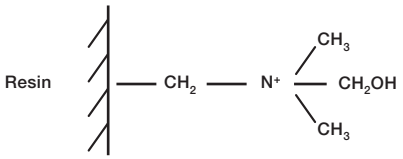


Fig. 1. AG 1 and AG 2 Resin functional groups.

Bead Properties

Property	Description
Type of ion exchanger	Strong anion
Functional group	See Figure 1
Minimum wet capacity	2% crosslinkage: 0.6 meq/ml 4% crosslinkage: 1 meq/ml 8% Crosslinkage: 1.2 meq/ml
Nominal density	2% crosslinkage: 0.65 g/ml 4% crosslinkage: 0.7 g/ml 8% crosslinkage: 0.75 g/ml
Recommended linear flow rate based on application	Removing trace ions : 5–10 cm/min Separations with very few components: 1–3 cm/min Separations of multicomponent samples: 0.3–1.0 cm/min Using high resolution resins with small particle size: 1–2 cm/min
Pressure vs. flow performance	See Table 1
pH stability	0–14
Shipping solution	Wet mesh
Regeneration	Equilibrate in correct ionic form; IEX followed by neutralization
Sanitization	1 M NaOH, 70% ethanol. Autoclave except OH form; OH form sanitization not required
Storage conditions	Ambient temperatures
Chemical stability	Acid, base, organic solvents; see bulletin LIT212
Shelf life	3–5 years

Pressure/Flow Performance

The flow rate in a chromatography column increases with increasing particle size. However, the attainable resolution increases with decreasing particle size and narrower size distribution ranges. Particle size is given in either mesh size or micron size as shown in Table 1. The larger the mesh size number, the smaller the particle size.

Table 1. Wet mesh and equivalent micron diameters.

Wet Mesh (U.S. Standard)											
16	20	40	50	80	100	140	200	270	325	400	
Micron Diameter (1 µm = 0.001 mm)											
1,180	850	425	300	180	150	106	75	53	45	38	

Large mesh material (20–50 and 50–100 mesh) is used primarily for preparative applications and batch operations where the resin and sample are slurried together. Medium mesh resin (100–200) may be used in batch as well as column applications. Medium mesh is an ideal general purpose particle size for use in analytical- and preparative-scale column chromatography. Fine mesh material (200–400 and –400 mesh) is used for high-resolution analytical separations.

Technical Description

AG 1 Resins that have lower crosslinkages (for example, AG 1-X2) are useful for higher molecular weight molecules, such as peptides, nucleotides, and uranium complexes. Resins with higher crosslinkages (for example, AG 1-X8) are useful for low molecular weight inorganic anions and for applications such as cyclic nucleotide assays and fractionation of organic acids.

AG 2 Resins are similar to AG 1 Resins but are less basic and less resistant to oxidation. AG 2 Resins are capable of separating sugars, sugar alcohols, and gluocosides. The isomerization of some sugars, which occurs with AG 1 Resins, does not occur with AG 2 Resins. Table 2 shows the approximate molecular weight exclusion limits in water for resins of various crosslinkages.

The resins are best used for the separation of low molecular weight (<3,000) compounds, including inorganic ions, organic acids, nucleic acids, carbohydrates, and metal ions. They can be used for nitrate removal, purification of sugar compounds, and pharmaceutical and fermentation applications.

Table 2. Approximate molecular weight exclusion limits for ion exchange resins in water.

Percent Crosslinking	Approximate MW Exclusion Limit for Globular Molecules
2%	2,700
4%	1,400
8%	1,000
10%	800
12%	400

In an IEX procedure, the counterions on the resin are replaced by sample ions that have the same charge. With AEX resins such as AG 1, neutral species and cations do not interact with the resin. In the chloride form of AG 1 and AG 2 Resin, the counterion on the resin is Cl^-. A resin can be converted from one ionic form to another. Usually the resin is used in an ionic form with a lower selectivity for the functional group than the sample ions to be exchanged. The sample ions are then exchanged onto the resin when introduced, and can be eluted by introducing an ion with higher affinity for the resin or a high concentration of an ion with equivalent or lower affinity. Table 3 shows the relative selectivity of various counterions. In general, the lower the selectivity of the counterion, the more readily it exchanges for another ion of like charge. The order of selectivity can also be used to estimate the effectiveness for different ions as eluants, with the most highly selective being the most efficient. Finally, the order of selectivity can be used to estimate the difficulty of converting the resin from one form to another. Conversion from a highly selected to a less highly selected form requires an excess of the new ion.

Table 3. Relative selectivity of various counterions.

Counterion	Relative Selectivity for AG 1 Resin	Relative Selectivity for AG 2 Resin
OH^-	1.0	1.0
Benzene sulfonate	500	75
Salicylate	450	65
Citrate	220	23
I^-	175	17
Phenate	110	27
HSO_4^-	85	15
ClO_3	74	12
NO_3	65	8
Br^-	50	6
CN^-	28	3
HSO_3^-	27	3
BrO_3^-	27	3
NO_2^-	24	3
Cl^-	22	2.3
HCO_3^-	6.0	1.2
IO_3^-	5.5	0.5
HPO_4^-	5.0	0.5
Formate	4.6	0.5
Acetate	3.2	0.5
Propionate	2.6	0.3
F^-	1.6	0.3

Publications

Application	Resin	Reference
Recovery of Pi from glucose-phosphate	AG 1-X4	Stroop SD and Boyer PD (1985). Biochem 24, 2304.
Extraction of 5-hydroxy-indole acetic acid from CSF and urine	AG 1-X8	Dombro RS and Hutson DG (1980). Clin Chim Acta 100, 231.
Anion removal from porphyrin in urine	AG 1-X8	Torben K and Penderson JS (1978). Scand J Clin Lab Invest 38, 279.
Purification of cyclic and nucleotides	AG 1-X8	Shanfield J et al. (1981). Anal Biochem 113, 256.
Purification of carboxylated pepsinogen	AG 1-X8	Rajagopalan TG et al. (1966). J Biol Chem 241, 4940.
Separation of cAMP from cGMP	AG 1-X8	Kuehl FA Jr et al. (1974). Proc Nat Acad Sci USA 71, 1866; Fallon AM and Wyatt GR (1975). Anal Biochem 63, 614.
Concentration of amines	AG 1-X8	Minkler PE et al. (1984). J Chromatog 336, 271.
Removal of triiodide	AG 1-X8	Basciano LK et al. (1986). J Biol Chem 261, 11823.
Concentration of niacin prior to HPLC analysis	Ag 1-X8	Tyler TA and Shrago RR (1980). J Liq Chromatog 3, 269.
Removal of organic acids and carbohydrates from guanidino compounds	AG 1-X8	Marescau B et al. (1986). J Chromatog 377, 334.
Removal of hormone from serum	AG 1-X8	Stanley F et al. (1966). J Biol Chem 261, 9400.
	AG 2-X8	Stringer BMJ and Wynford Thomas D (1982). Hormone Res 16, 392.
Concentration of phytate	AG 1-X8	Ellis R and Morris ER (1986). Cereal Chem 63, 58.
Removal of ATP from proteoliposomes	AG 1-X8	Woldegiorgis G and Shrago E (1985). J Biol Chem 260, 7585.
Removal or concentration of organic acids	AG 1-X8	Chen PM et al. (1982). J Amer Soc Hort Sci 107, 807.

Metal Separation on Anion Exchangers

Metals	Recommended Resin	Eluant and Eluted Ions	Reference
Ni, Mn (ii), Co (ii), Cu (ii), Fe (iii), Zn (ii)	AG 1-X8	Ni - 12 M HCl: Mn - 6 M HCl; Cu - 2.5 M HCl; Fe - 0.5 M HCl; Zn - 0.005 M HCl	Kraus KA and Moore GE (1953). J Amer Chem Soc 75, 1460.
Ni, Co, Cu, Zn	AG 1-X8	Ni - 96% MeOH, 0.2 M HCl; Co - 55% IPA, 1.3 M HCl; Cu - 55% IPA, 0.1 M HCl; Zn - 0.005 M HCl	Fritz JS et al. (1961). Talanta 8, 143.
Mn, Co, Ni Fe, Mo, (also Cr, Zn, Cd, Hg)	AG 1-X8	Mn, Co, Ni - 8.5 x 10^{-2} M tartrate; Fe - tartaric acid in 0.1 M HCl; Mo - 3 M NaOH	Morie GP and Sweet TR (1964). J Chromatog 16, 201.
Th, Hf, Zr, Mo	AG 1-X8	Th - 0.7 N H_2SO_4; Hf - 1.25 N H_2SO_4; Zr - 2.0 N H_2SO_4; Mo - 2.0 N NH_4; NO3, 0.5 N NH_3	Strelow FWE and Bothma CJC (1967). Anal Chem 39, 595.
V, Th, Fe	AG 1-X8	Absorbed as citrate complexes; Th - 8 M HCl; Fe - IBMK, acetone, 1 N HCl (1:8:1 v/v); V - 1 M HCl	Korkisch J and Krivanec H (1976). Anal Chim Acta 83, 111.
Bi, Pb, Cd, Zn	Ag 1-X8	Pb, Cd,Zn - -HNO3; Bi - EDTA	Strelow FWE (1978). Anal Chem 50, 1359.

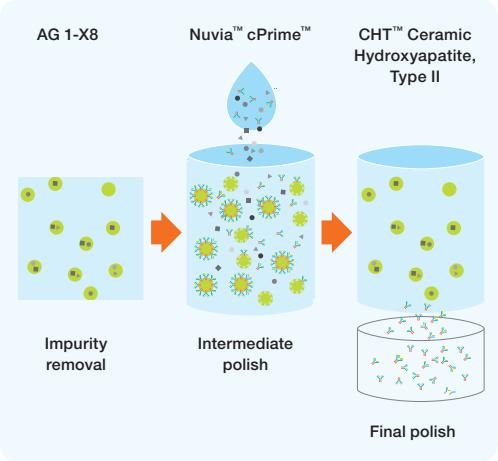
Peptide and Protein Separations on Anion Exchangers

Application	Resin	Reference
Separation of small peptides from rabbit muscle	AG 1-X2	Titani K et al. (1978). Biochem 17, 5680.
Separation of peptides from horse liver cytochromes	AG 1-X2	Ozuls J et al. (1976). J Biol Chem 251, 6767
Purification of fungal glucoamylase	AG 1-X4	Bhella RS and Altosaar I (1984). Anal Biochem 140, 200.

AEX Resins in Enzymatic Assays

Enzyme	Substrate	Product	Resin	Reference
NADase	NAD	Nicotinamide	AG 1-X2	Moss J et al. (1976). Proc Nat Acad Sci USA 73, 4424.
Cyclic 3',5'-nucleotide phosphodiesterase	cAMP	Adenosine	AG 1-X2	Brooker G et al. (1968). Biochem 12, 4177; Ong KK and Rennie PIC (1976). Anal Biochem 76, 53; Thompson WJ et al. (1978). Advan Cyclic Nucleotide Res 9, 69.
Sucrose synthetase; sucrose phosphate synthetase	UDP-glucose and fructose; UDP-glucose and fructose-6-P	Sucrose + UDP; UDP + sucrose-6-P	AG 1-X4	Salerno GL et al. (1979). Anal Biochem 93, 196.
Guanylate cyclase	GTP	cGMP	AG 1-X8; neutral alumina	Krishnan N and Krishna G (1976). Anal Biochem 70, 18.
Hexokinase	Mannose	Mannose-6-P	AG 1-X8	Li E et al. (1978). J Biol Chem 253, 7762.
Choline kinase	ACh + ATP	Phosphorylcholine	AG 1-X8	Kato AC et al. (1975). Can J Physiol Pharmacol 53, 1050.
HMG-CoA reductase	HMG-CoA	Mevalonolactone	AG 1-X8	Edwards PA et al. (1979). J Lipid Res 20, 40.
Glutamine synthetase	Glutamate	Glutamine	AG 1-X8	Pishak MR and Phillips AT (1979). Anal Biochem 94, 88.

Purification Workflow



Ordering Information

Catalog #	Ionic Form	Dry Mesh Size	Nominal Capacity, meq/ml	Wet Bead Diameter, µm	Nominal Density, g/ml	Package Size, kg
Bulk Resins						
AG 1-X2 Resin, Analytical Grade						
140-1231	Chloride	50–100	0.6	180–500	0.65	0.5
140-1241	Chloride	100–200	0.6	106–250	0.65	0.5
140-1251	Chloride	200–400	0.6	75–180	0.65	0.5
140-1253	Acetate	200–400	0.6	75–180	0.65	0.5
140-1255	Acetate	200–400	0.6	75–180	0.65	10
140-1256	Acetate	200–400	0.6	75–180	0.65	1
AG 1-X2 Resin, Biotechnology Grade						
1431255	Hydroxide	200–400	0.6	75–180	0.65	0.1
AG 1-X4 Resin, Analytical Grade						
140-1331	Chloride	50–100	1.0	180–425	0.70	0.5
140-1341	Chloride	100–200	1.0	106–250	0.70	0.5
140-1342	Chloride	100–200	1.0	106–250	0.70	10
140-1351	Chloride	200–400	1.0	63–150	0.70	0.5
AG 1-X4 Resin, Biotechnology Grade						
1431345	Hydroxide	100–200	1.0	63–150	0.70	0.1
AG 1-X8 Resin, Analytical Grade						
140-1421	Chloride	20–50	1.2	300–1,180	0.75	0.5
140-1422	Hydroxide	20–50	1.2	300–1,180	0.75	0.5
140-1424	Hydroxide	20–50	1.2	300–1,180	0.75	10
140-1425	Chloride	20–50	1.2	300–1,180	0.75	1
140-1426	Chloride	20–50	1.2	300–1,180	0.75	10
140-1431	Chloride	50–100	1.2	180–425	0.75	0.5
140-1432	Chloride	50–100	1.2	180–425	0.75	10
140-1441	Chloride	100–200	1.2	106–180	0.75	0.5
140-1443	Acetate	100–200	1.2	106–180	0.75	0.5
140-1444	Formate	100–200	1.2	106–180	0.75	0.5
140-1445	Chloride	100–200	1.2	106–180	0.75	10
140-1446	Acetate	100–200	1.2	106–180	0.75	10
140-1447	Acetate	20–50	1.2	106–180	0.75	10
140-1451	Chloride	200–400	1.2	45–106	0.75	0.5
140-1453	Acetate	200–400	1.2	45–106	0.75	0.5
140-1454	Formate	200–400	1.2	45–106	0.75	0.5
140-1456	Chloride	200–400	1.2	45–106	0.75	10
140-1457	Chloride	100–200	1.2	45–106	0.75	5
141-1842	Chloride	100–200	1.2	106–180	0.75	10
141-1853	Nitrate	200–400	1.2	45–106	0.75	10
AG 1-X8 Resin, Biotechnology Grade						
1432445	Hydroxide	100–200	1.2	106–180	0.75	0.1
1432446	Hydroxide	200–400	1.2	45–106	0.75	0.1
AG 2-X8 Resin, Analytical Grade						
140-2421	Chloride	20–50	1.2	300–1,180	0.75	0.5
140-2423	Chloride	20–50	1.2	300–1,180	0.75	10


MIXED MODE

SPECIALTY BEADS

AG® 501

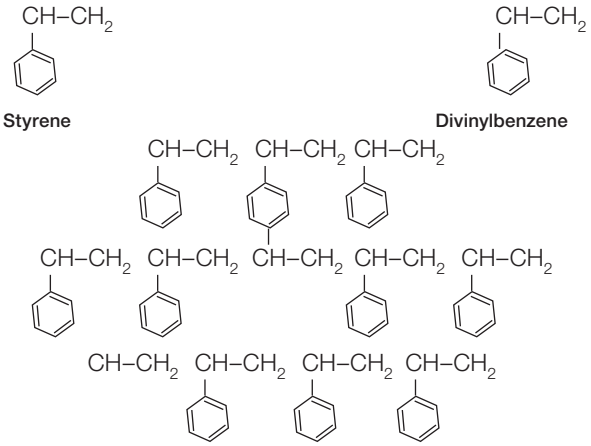
FUNCTIONALITY
- Deionizing water, urea, acrylamide, formamide, glyoxal, or PEG
- Condensate polishing
- Ultrapurification of buffers and ionic reagents

AG 501 Resins are mixed-bed resins containing equivalent amounts of AG 50W-X8, a strong CEX resin (H^+ form), and AG 1-X8, a strong AEX resin (OH^- form). They are based on a styrene divinylbenzene copolymer matrix. Mixed-bed resins are used for deionizing water or other nonionic reagents such as urea, acrylamide, formamide, latex particles, or glyoxal. Deionization is the complete removal of all ionic species from a solution. For additional details about AG 1 and AG 50 Resins, see pages 70–79.

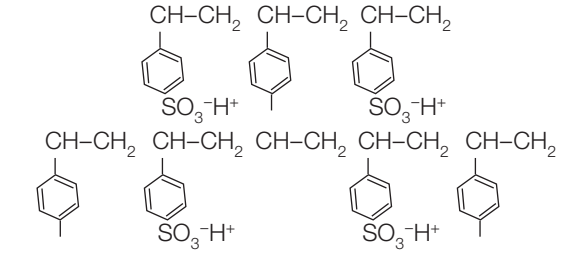
Benefits

- Mixed-bed ion exchanger
- Extensively purified to remove impurities
- Rapid, simple deionization of solutions
- Available with blue dye that functions as a visual indicator

A. Crosslinked styrene-divinylbenzene copolymer



B. AG 50W crosslinked strongly acidic CEX resin



C. AG 1 crosslinked strongly basic AEX resin

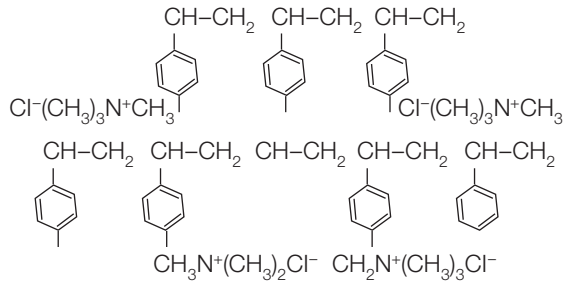


Fig. 1. Molecular structures of styrene-divinylbenzene crosslinked copolymers with attached groups for AG 50W and AG 1 Resins.

Bead Properties

Property	Description
Type of ion exchanger	Strong anion and cation (mixed-bed resin)
Functional group	H^+ and OH^-
Minimum wet capacity	8% crosslinkage: 1.8 meq/ml
Particle diameter	Mesh size 20–50: 300–1,180 µm Mesh size 25–35 : 500–700 µm
Nominal density	0.75 g/ml
pH stability	0–14
Shipping solution	Wet mesh
Regeneration	See Regeneration section
Sanitization	Resins are stable in acid, base, and organic solvents and may be autoclaved, except the OH form
Storage conditions	Ambient temperatures
Chemical stability	Acid, base, organic solvent
Shelf life	Mixed-bed ion exchange resins are stable for two years when stored at ambient temperatures and protected from exposure to ultraviolet light. The shelf life may be extended by storing the resin at 4°C

Applications

AG 501 Mixed-Bed Resins can be used to prepare nonionic reagents for critical analytical applications. Either the batch or column method may be used to obtain purified urea, acrylamide, formamide, glyoxal, or PEG, although the batch technique is much more common. Industrial applications include water softening, waste treatment in nuclear power plants to remove radioactive substances from water, and condensate polishing. Table 1 provides guidelines for column deionization for a range of volumes.

Table 1. Guidelines for column deionization.

Column Size, cm (diameter x length)	Weight of Resin, g	Deionized Water*, L (approximate)
0.5 x 20	2.6	1.5
1.0 x 50	27.0	6.7
1.5 x 100	118.0	29.0
2.5 x 100	325.0	80.0
5.0 x 100	1,300.00	325.0
15.0 x 120	13.6	3,500.0

* Based on using 100 ppm NaCl. This volume will vary depending on initial water quality.

Regeneration

Mixed-bed resins used in laboratory-scale applications are not normally regenerated because of the difficulty in separating the mixed anion and cation resins, the large volumes of regenerants required for the anion resin, and difficulty in accurately remixing chemically equivalent resins. See bulletin LIT205 for the regeneration protocol, which is cost effective for large-scale applications.

AG 501-X8(D) Resin has a blue dye irreversibly bound to the anion exchange resin, which turns from blue to gold when the exchange capacity is exhausted, indicating regeneration is necessary.

Publications

Kapp OH and Vinogradov SN (1978). Removal of sodium dodecyl sulfate from proteins. Anal Biochem 91, 230–235.

Bakker JA et al. (1981). Evidence for carrier ampholyte-peptide interactions during somatomedin purification. J Chromatogr 209, 273–282.

Brown WD and Green S (1970). Removal of proteins from isoelectric focusing media. Anal Biochem 34, 593–595.

Ordering Information

Catalog #	Description	Mesh Size	Diameter, µm	Nominal Density, g/ml	Package Size, kg
Bulk Resins					
142-6424	**AG 501-X8 Resin**	20–50	300–1,180	0.75	0.5
142-6427	**AG 501-X8 Resin**	20–50	300–1,180	0.75	10
1437424	**AG 501-X8 Resin, biotechnology grade**	20–50	300–1,180	0.75	0.1
143-7428	**AG 501-X8 Resin, analytical grade**	20–50	300–1,180	0.75	10
1436424	**AG 501-X8 Resin, molecular biology grade**	20–50	300–1,180	0.75	0.1
143-6426	**AG 501-X8 Resin, molecular biology grade**	20–50	300–1,180	0.75	10
142-6425	**AG 501-X8(D) Resin**	20–50	300–1,180	0.75	0.5
1437424	**AG 501-X8 Resin, biotechnology grade**	20–50	300–1,180	0.75	0.1
1437425	**AG 501-X8(D) Resin, biotechnology grade**	20–50	300–1,180	0.75	0.1
143-7428	**AG 501-X8 Resin, biotechnology grade**	20–50	300–1,180	0.75	10
1436425	**AG 501-X8(D) Resin, molecular biology grade**	20–50	300–1,180	0.75	0.1
143-6427	**AG 501-X8(D) Resin, analytical grade**	20–50	300–1,180	0.75	10
142-7425	**Bio-Rex™ MSZ 501(D)**	25–35	50–700	0.75	0.5
142-7426	**Bio-Rex MSZ 501(D)**	25–35	50–700	0.75	10


SPECIALTY

SPECIALTY BEADS

Bio-Beads™ SM-2 Resin

FUNCTIONALITY
- Absorption of nonpolar substances
- Removal of detergents such as Triton X-100
- Removal of organics
- Cleanup of drugs from plasma and urine
- Extraction of dyes and mycotoxins from food products

Nonpolar polystyrene Bio-Beads SM-2 Adsorbents are particularly useful for the adsorption of nonpolar substances or surface active agents from aqueous solutions. The adsorbent has a high surface area and uniform pore size. Bio-Beads SM-2 can be used with a variety of solvents, including alcohols, petroleum ether, diethyl ether, hexane, solvent mixtures, and aqueous media.

Benefits

- Neutral macroporous polymeric beads
- High surface area for adsorbing organics
- Used in aqueous solutions with solvents and/or including detergents without expansion or contraction
- Useful for quantitative removal of surfactants from aqueous feedstreams

Bead Properties

Property	Description
Type of function	Hydrophobic
Pore diameter (dry bead)	90 Å
Capacity	0.07 g Triton X-100/g
Wet density	1.02 g/cc
Recommended linear flow rate based on application	Batch mode or column method not exceeding 82 bar
Pressure limitations	82 bar (1,200 psi)
Temperature limit	250°C
pH stability	0–14
Shipping solution	Dry
Regeneration	4 bed volumes of methanol, followed by rinsing in deionized water
Sanitization	Resin can be autoclaved
Storage conditions	Short term (1 week): use the elution solvent Long term: 0.05% sodium azide solution or 20% methanol or isopropanol
Shelf life	5 years

Applications

Bio-Beads SM-2 Adsorbents are used for analysis of organic compounds, drugs, biological, foods, pesticides, chemicals, metals, and detergents. The following compounds can be concentrated or separated by Bio-Beads SM Adsorbents:

- Trace organics
- Morphine
- PAH compounds
- Methaqualone hydrocarbons and PCBs
- Sulfas
- Aminocarb insecticides
- Free rhodamine
- Carbamate insecticides
- Prostaglandins
- Ethyl and methyl parathion
- Steroids
- Metals
- Bile acids
- Carboxylic acids
- Hormones
- Phenolic acids
- Purines and pyrimidines
- Flavonoids
- Acid dyes
- Mycotoxins
- Naringin and limonin
- Proline and hydroxyproline
- Detergents

Publications

Food

Application	Reference
Flavonoids from aqueous fractions	Rosler KH and Goodwin RS (1983). J Natural Products 47, 188.
Acid dyes	Uematsu T et al. (1979). J Chromatogr 172, 327.
Naringin and limonin from grapefruit juice	Chandler BV and Johnson RI (1981). Proc Int Soc Citriculture 2, 885.
Triadimefon in grape wine	Nickless G and Spitzer T (1981). J Chromatogr 208, 409.

Detergents

Application	Reference
Triton X-100 detergent	Holloway PW (1973). Anal Chem 53, 304; Drexler G et al. (1986). J Immun Methods 95, 117; Bonomi F and Kurtz DM Jr (1984). Anal Biochem 142, 226; Welling GW et al. (1984). J Chromatogr 297, 101; Metsikko K et al. (1986). The EMBO Journal 5, 3429.
Cholate detergent	Bonomo EA and Swaney JB (1988). J Lipid Research 29, 380; Jinks DC and McElhaney RN (1987). Anal Biochem 164, 331.
Deoxycholate	Horigome T and Sugano H (1983). Anal Biochem 130, 393; Lorusso DJ and Green FA (1974). Science 188, 66; Shechter I and Bloch K (1971). J Biol Chem 246, 7690.
NP-40 detergent	Momoi T (1979). Biochem Biophys Res Commun 87, 541.
Emulgen 911	Gibson GG and Schenkman FB (1978). J Biol Chem 253, 5957; Warner M (1982). J Biol Chem 257, 12995.
Emulphogene BC-720	Brunch RC et al. (1986). J Biol Chem 261, 9450.
Coating microtiter plates with detergent soluble membrane proteins	Drexler G et al. (1986). J Immunological Methods 95, 117.

Pesticides

Application	Reference
Aminocarb insecticide from water	Levesque D and Mallet VN (1983). Intern J Environ Anal Chem 16, 139.
Carbamate insecticides	Sundaram KMS et al. (1979). J Chromatogr 177, 29.
Chlorinated pesticides	McNeil EE and Otsen R (1977). J Chromatogr 132, 277.
Ethyl and methyl parathion	Paschal DD et al. (1977). Anal Chem 49, 1551.
Fenitrothion and its degradation products	Volpe G and Mallet VN (1981). Chromatographia 14, 333.
Organophosphorous pesticides	LeBel GL et al. (1979). J Assoc Off Anal Chem 62, 241.

Ordering Information

Catalog #	Description
Bulk Resin	
1523920	**Bio-Beads SM-2 Adsorbents**, 20–50 mesh, 100 g
1528920	**Bio-Beads SM-2 Adsorbents**, biotechnology grade, 20–50 mesh, 25 g
152-3922	**Bio-Beads SM-2 Adsorbents**, 20–50 mesh, 1 kg
152-3923	**Bio-Beads SM-2 Adsorbents**, 20–50 mesh, 10 kg


SPECIALTY

SPECIALTY BEADS

Bio-Beads™ S-X Resin

FUNCTIONALITY

- Quantification of pesticides and rodenticides
- Measurement of organic priority pollutants in sludge
- Separation of polycyclic aromatic compounds
- Assessment of tissue reaction to biomaterial
- Fractionation of halogenated environmental contaminants
- Separation of tall oil components, lipids, alkalines, 21 fatty acids, hydrocarbons, and polystyrenes

Bio-Beads S-X Beads are a series of porous crosslinked polystyrene polymers used for size exclusion separations of lipophilic polymers and low molecular weight hydrophobic materials in the presence of organic solvents. These nonaqueous spherical beads are used in a similar manner to aqueous gels except that they are swollen with organic solvents during the separation process.

The beads in the Bio-Beads S-X series have exclusion limits from 400 to 14,000 daltons. This range makes them particularly suitable for the fractionation and separation of low molecular weight organic polymers and other hydrophobic substances. The amount of divinylbenzene crosslinkage determines the pore size, and hence the molecular weight exclusion limit of a particular gel in this series. The beads are available with crosslinkages from 1–12%.

Pore dimensions and exclusion limits are also influenced by the eluant employed; maximal expansion of the matrix is achieved with relatively nonpolar aromatic solvents. The beads are typically used with benzene, toluene, xylene, carbon tetrachloride, and mixtures of solvents.

Gel filtration is the mode of separation that occurs with Bio-Beads S-X Beads. Each Bio-Beads S-X series has a molecular weight range within which molecules can be separated. The large compounds, greater than the molecular exclusion limit of each series, pass through the column unhindered, whereas small compounds, within the molecular weight operating range, will be retained in the column. The small compounds permeate the pores of the Bio-Beads S-X Beads, and thus take longer to pass through the column. This mechanism requires an eluant that is mobile; therefore, Bio-Beads S-X Beads must always be used in column mode.

Bead Properties

Property	Description
Type of function	Gel filtration/size exclusion of lipophilic polymers or solutes that require organic solvents
Mesh size/exclusion limit	1% crosslinkage: 200–400/ 600–14,000 MW 3% crosslinkage: 200–400/up to 2,000 MW 8% crosslinkage: 200–400/up to 1,000 MW 12% crosslinkage: 200–400/up to 400 MW
Swollen bed volume	1% crosslinkage: 7.5 ml/g benzene 3% crosslinkage: 4.75 ml/g benzene 8% crosslinkage: 3.1 ml/g benzene 12% crosslinkage: 2.5 ml/g benzene
Nominal density	2% crosslinkage: 0.65 g/ml 4% crosslinkage: 0.7 g/ml 8% crosslinkage: 0.75 g/ml
Recommended linear flow rate based on application	1% crosslinked resins: gravity flow 3% crosslinked resins: 5 ml/min at 20 bar (300 psi) 8–12% crosslinked resins: 33 bar limit (500 psi)
pH stability	0–14
Shipping solution	Dry
Regeneration	To regenerate the resin, swell it to its maximum with a solvent such as methylene chloride, toluene, or tetrahydrofuran
Sanitization	Resin can be autoclaved
Storage conditions	Ambient temperatures
Chemical stability	Bio-Beads S-X Beads are highly chemically resistant, though explosive mixtures may form in the presence of strong oxidizing reagents such as chromic acid, nitric acid, and hydrogen peroxide
Shelf life	5 years

Performance

Bio-Beads S-X2 and S-X8 Beads have been used in multicolumn systems for separating various components of tall oil, wood resin, and gum resin by gel permeation chromatography. Figure 1 shows the fractionation of tall oil with Bio-Beads S-X Beads. The injected acid sample was fully recovered and no delay in elution time was noticed.

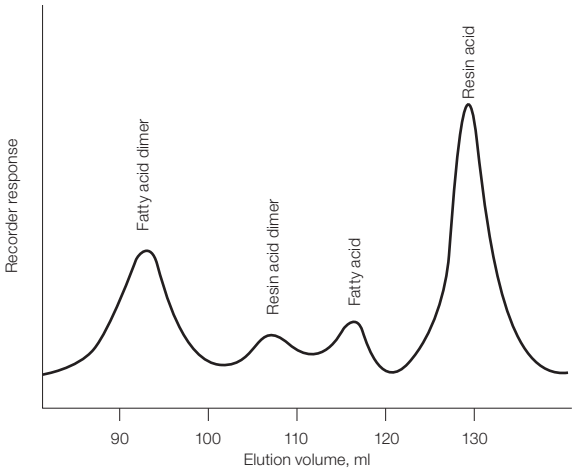


Fig. 1. Separation of tall oil

A variety of lipophilic polymer substances has been successfully purified on beds of Bio-Beads S-X Beads (Figure 2).

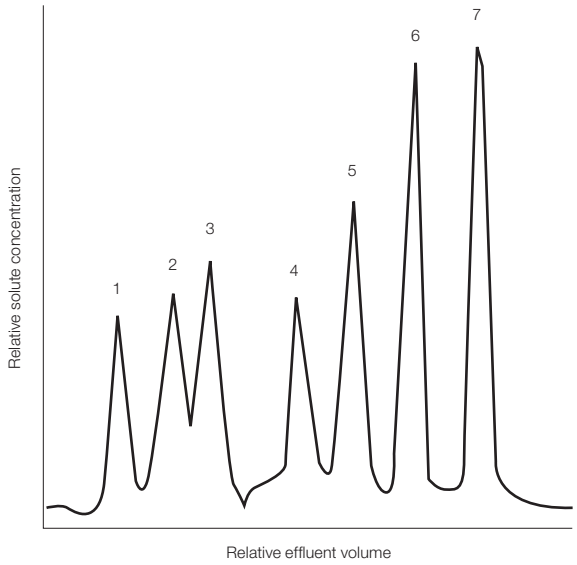


Fig. 2. Separation of triglycerides and hydrocarbons on Bio-Beads S-X1 and S-X2 Beads in benzene (two beds in series). 1, tristearin; 2, trimyristein; 3, trilaurin; 4, tricaprylin; 5, tricaproin; 6, hexadecane; 7, undecane.

Applications

Many different types of compounds can be separated on Bio-Beads S-X Beads. The beads can be used for analysis and quantification of pesticides and rodenticides. Bio-Beads S-X Beads are the basis of the official EPA procedure for the measurement of organic priority pollutants in sludge. The beads are useful for separation of polycyclic aromatic compounds, assessment of tissue reaction to biomaterial, and fractionation of halogenated environmental contaminants. Other applications include separation of tall oil components, lipids, alkalines, fatty acids, a variety of hydrocarbons, and polystyrenes. These beads can also be used to determine polymer molecular weights and molecular weight distributions. They have been used for the separation of low molecular weight trimethylsilylated silicic acid and for the isolation of low molecular weight polar organics in fatty tissues for subsequent GLC-MS analysis. They have also been used for the analysis of fish lipid extracts and for fractionation of food-grade PVC resins.

Publications

Application	Reference
Pesticides	Stalling DL et al. (1972). JAOAC 55, 32; Johnson LD et al. (1976). JAOAC 59, 174; Steinwandter H and Fresenius Z (1982). Anal Chem 313, 536; Blaha JJ and Jackson PJ (1985). JAOAC 68, 1095.
Rodenticides	Hunter K (1984). J Chromatogr 299, 405; Hunter K (1985). J Chromatogr 321, 255.
Sludge	Haile CL and Lopez-Avila V (1984). U.S. Environmental Protection Agency, Project Summary #600/S4-84-001.
Polycyclic aromatics	Friley BK et al. (1983). J Chromatogr 258, 310; Chamberlain WJ et al. (1979). Anal Chim Acta 222, 235; Snook ME et al. (1975). Anal Chem 47, 1155.
Tissue reaction	Hood CI et al. (1984). J Biomed Materials Res 18, 1031; Schoen F et al. (1986). J Biomed Materials Res 20, 709.
Halogenated environmental contaminants	Stalling DL et al. (1979). ASTM Special Publication 686, 302; Musial C and Uthe JF (1986). JAOAC 69, 462; Ault JA et al. (1979). J Ag and Food Chem 27, 825; LeBel G and Williams DT (1986). JAOAC 69, 451.
Tall oil	Chang T-L (1968). Anal Chem 40, 989.
Lipids	Tipton CL et al. (1964). J Chromatogr 14, 486; Hirsch J (1960). Colloq Int Centre Nat Res Sci 99, 11.
Alkalines	Chang T-L (1967). Anal Chim Acta 39, 519.
Fatty acids	Chang T-L (1968). Anal Chim Acta 42, 51.
Hydrocarbons	Henfrickson JG (1968). J Chromatogr 32, 543.
MW determination	Cantow MJR et al. (1967). J Polymer Sci 5, 987; Coll H (1970). Separ Sci 5, 273; Pickett HE et al. (1966). J Applied Polymer Sci 10, 917; Cantow MJR et al. (1967). J Polymer Sci (Part C) 16, 13; Cantow MJR et al. (1967). J Polymer Sci (Part A-1) 5, 1391.
Trimethysilylated silicic acid	Shimono T et al. (1979). J Chromatogr 179, 323.
Polar organics in fatty tissues	Kuehl DH and Leonard EN (1978). Anal Chem 50, 182.
Fish lipid extracts	Burns BG et al. (1981). JAOAC 64, 282.
Food grade PVC fractionation	Gilbert J et al. (1985). J Chromatogr 320, 361; Waliszewski SM and Szymczynski GA (1985). J Chromatogr 321, 480.

Ordering Information

Catalog #	Description
Bulk Resin	
1522150	**Bio-Beads S-X1 Support**, 100 g
152-2151	**Bio-Beads S-X1 Support**, 1 kg
1522750	**Bio-Beads S-X3 Support**, 100 g
1523350	**Bio-Beads S-X8 Support**, 100 g
1523650	**Bio-Beads S-X12 Support**, 100 g

SPECIALTY BEADS

Chelex® 100 Resin

FUNCTIONALITY
- Removal of trace metals
- Concentration of glyphosate
- Removal of metals from buffers

Chelex 100 Chelating IEX Resins are styrene divinylbenzene copolymers containing iminodiacetate ions, which act as chelating groups in binding polyvalent metal ions. The carboxylic acid groups of Chelex 100 Resin classify it as a weak CEX resin, but it differs from other exchangers in this class as it features uniquely high selectivities for metal ions and much higher bond strengths. The resin can be used to ultrapurify buffers and ionic reagents; it will scavenge metal contaminants without altering the concentration of monovalent ions. Chelex 100 Resin has a stronger selectivity for copper, iron, and other heavy metal ions than monovalent cations such as sodium and potassium. Its selectivity for divalent over monovalent ions is approximately 5,000 to 1, and it has a strong attraction for transition metals, even in highly concentrated salt solutions. The selectivity of Chelex Resin for metal cations corresponds to that of iminodiacetic acid. The selectivity factor is a quantitative measure of the affinity that Chelex Resin displays for a particular cation compared to its affinity for a reference cation.

Benefits

- Strong selectivity for heavy metal ions
- Strong attraction for transition metals in high-salt solutions
- Extensively purified to remove impurities
- Sized to provide high resolution and reproducibility

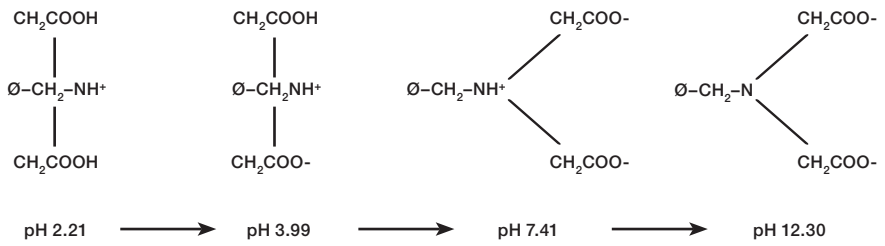


Fig. 1. Change in structure of Chelex Resin ligand with increasing pH.

The chelating Chelex Resin is efficiently regenerated in dilute acid and operates in basic, neutral, and weakly acidic solutions of pH 4 or higher. At very low pH, the resin acts as an anion exchanger. Figure 1 shows the zwitterionic forms of the Chelex Resin as a function of pH.

Bead Properties

Property	Description
Type of ion exchanger	Strong cation
Functional group	R-$CH_2N(CH_2COO^-)_2$
Particle size range	75–150 µm 150–300 µm 300–1,180 µm
Total ionic capacity	0.4 meq/ml (defined as $Cu(NH_3)_4^{2+}$ uptake)
Recommended linear flow rate	≥20 cm/min (separations of strong cations from weak cations) <4 cm/min (separations of similar species)
pH stability	2–14
Shipping solution	Dry powder
Regeneration	Two-step process: 1. Convert to the hydrogen form using acid 2. Convert to the desired ionic form using the hydroxide of the cation desired
Sanitization	0.5–1.0 N NaOH
Storage conditions	Store in salt form such as sodium or ammonium
Chemical stability	1 M acid, 1 M base, organic solvents, 8 M urea, reducing agents
Shelf life	5 years

Applications

Chelex 100 Resins can be used in many processes, including analysis of trace metals in natural waters, reagents, biochemicals, and physiological fluids, removal of trace metals from reagents, biochemicals, physiological fluids, culture media, soils, and enzyme systems, recovery of metals from process streams, and chromatography of closely related metals. Chelex 100 is usually used in a batch process.

Trace Metal Removal

Chelex Resin offers a rapid method for thoroughly removing trace metal contaminants that could have an effect on biological fluids or biological systems under study. A unique IEX resin that is more selective for multivalent metals than the standard CEX resins, Chelex Resin will scavenge multivalent metal ion contaminants without altering the concentration of nonmetallic ions. In most cases, neither column nor batch treatment with Chelex 100 Resin has any effect on protein concentration or enzyme activity. Where low protein recovery is a problem, the protein can be dialyzed against a buffer containing Chelex 100 Resin.

Glyphosate Concentration and Cleanup

Chelex 100 Resin may be used to concentrate glyphosate (N-(phosphonomethyl)glycine) when the resin is in its iron form. When environmental water or an aqueous extract of crops such as soybeans, grapes, cabbage, or alfalfa is applied to Chelex 100 Resin (100–200 mesh, iron form), glyphosate forms a complex with the iron form of the resin. The glyphosate may subsequently be eluted with 6 M HCl and applied to AG® 1-X8 Resin (200–400 mesh, chloride form) for AEX cleanup. The glyphosate, along with its breakdown product aminomethyl phosphonic acid (AMPA), may then be quantified with an Aminex® Column. For more information, see bulletin LIT237.

Metal Analysis

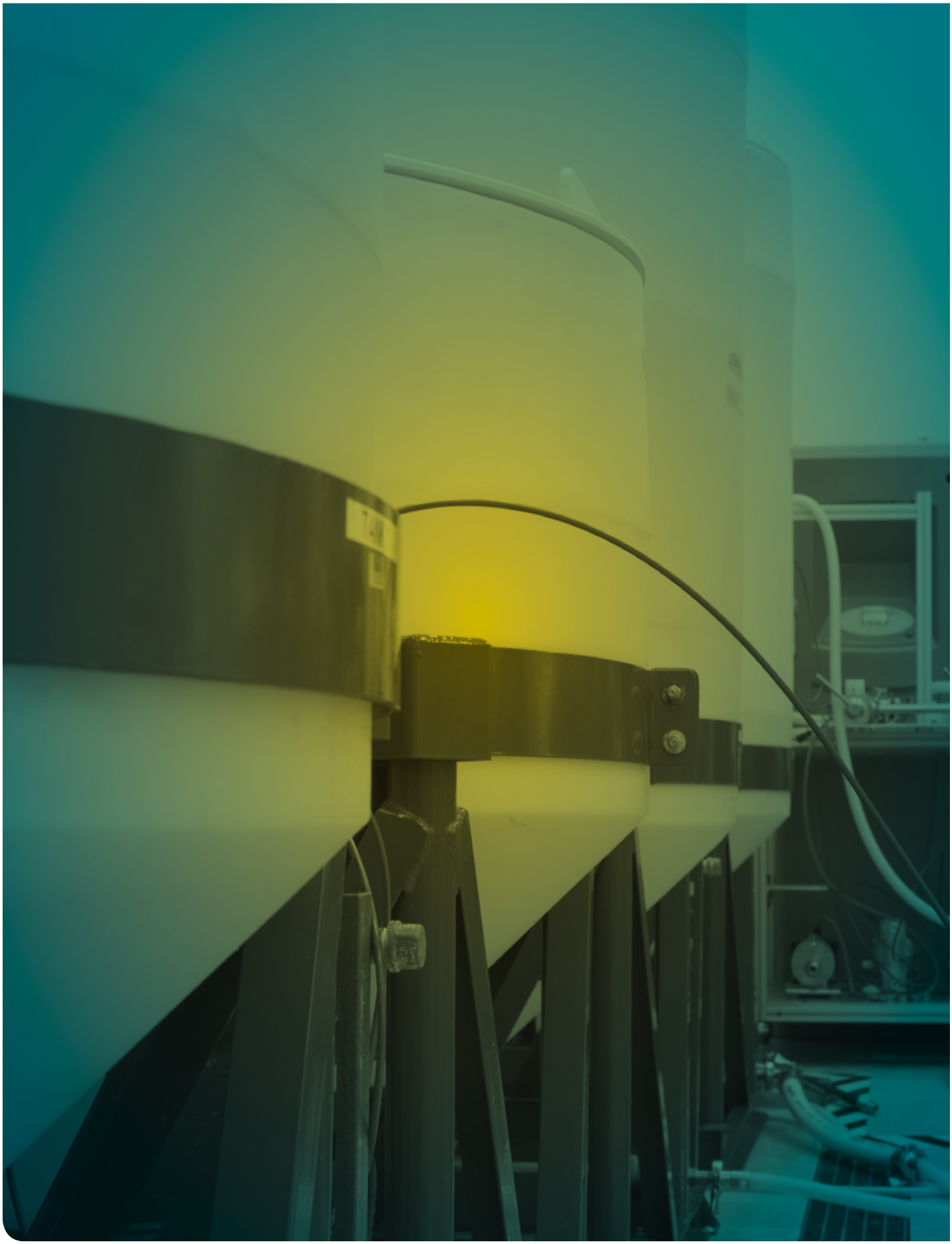
Trace metals can be concentrated by adsorption to Chelex Resin. For recommended use, see bulletin LIT200.

Publications

Application	Reference
Preparation of metal-free apoenzyme	O'Keefe ET et al. (1980). Biochem 19, 4954.
Removal of calcium from sarcoplasmic reticulum vesicles	Chiesi M and Inesi G (1980). Biochem 19, 2912.
Removal of extraneously bound metal ions from enzymes prior to NMR and ESR studies	Barker R et al. (1979). Biochem J 177, 289.
Batch removal of calcium from whole blood	Raymond FA and Weinshilboum RM (1975). Clin Chim Acta 58, 185.
Removal of metals from ATP	Sontheimer GM et al. (1987). Biochem 26, 2701.
Removal of metals from buffer, brine, and biological solutions	Knapp G et al. (1987). J Anal Atomic Spectrometry 2, 611; Laue TM et al. (1989). Biochem 28, 4762.
Removal of metals from S100b and melittin	Baudier J et al. (1987). Biochem 26, 2886.
Purification of dinucleotides	Reinhardt CG and Krugh TR (1978). Biochem 17, 4845.
Removal of metals from cell suspension	Bosron WF et al. (1975). Biochem 14, 2275.
Batch removal of metals from urine	Agarwal M et al. (1975). Anal Chem 47, 924.
Removal of metals from enzyme solutions	Dunn MF et al. (1980). Biochem 19, 718.
Removal of metals from guinea pig complement	Amiraian K et al. (1974) Immunology 26, 1135.
Removal of aldehydes and peroxides from polyethylene glycol	Ray WJ and Pavathingal JM (1985). Anal Biochem 146, 307.
Removal of interfering components from myo-inositol	Brandt SJ et al. (1985). Proc Nat Acad Sci 82, 3277.
Calcium removal from protein kinases	Putnam-Evans CL et al. (1990). Biochem 29, 2488.
Removal of divalent cations from NMR stock solutions	Devlin CC and Grisham CM (1990). Biochem 29, 6192; Chuknyisky PP et al. (1990). Biochem 29, 5987.

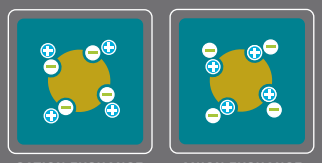
Ordering Information

Catalog #	Description
Bulk Resin	
142-2822	**Chelex 100 Resin**, analytical grade, 50–100 mesh, sodium form, 500 g
142-2832	**Chelex 100 Resin**, analytical grade, 100–200 mesh, sodium form, 500 g
1422825	**Chelex 100 Resin**, analytical grade, 100–200 mesh, iron form, 100 g
142-2842	**Chelex 100 Resin**, analytical grade, 200–400 mesh, sodium form, 500 g
1432832	**Chelex 100 Resin**, biotechnology grade, 100–200 mesh, sodium form, 100 g
142-2823	**Chelex 100 Resin**, 50–100 mesh, 10 kg
142-2834	**Chelex 100 Resin**, 100–200 mesh, 10 kg
142-2826	**Chelex 100 Resin**, 100–200 mesh, iron form, 10 kg
142-2827	**Chelex 100 Resin**, 100–200 mesh, iron form, 1 kg
142-2843	**Chelex 100 Resin**, 200–400 mesh, sodium form, 10 kg



CHAPTER 2

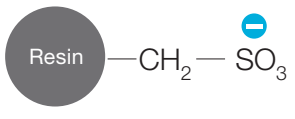
Functional Groups / Applications



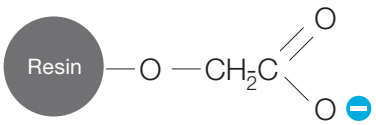
FUNCTIONAL GROUPS / APPLICATIONS

Ion Exchange Chromatography

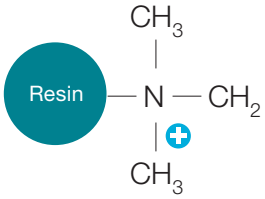
For 60 years, IEX has played a major role in the separation and purification of biomolecules. Today, IEX remains one of the most frequently used techniques for purification of proteins, peptides, nucleic acids, and other charged biomolecules, offering high resolution and group separations with high loading capacity. The technique can be used to separate molecular species that have only minor differences in their charge properties, for example two proteins differing by one charged amino acid. These features make IEX well suited for capture and intermediate purification or polishing steps in a purification protocol, and the technique can be used from microscale purification and analysis through to purification of kilograms of product.



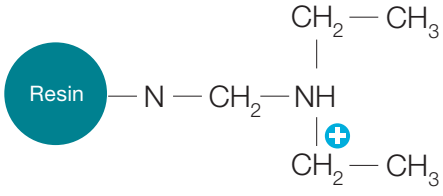
S-cation exchanger



CM-cation exchanger



Q-anion exchanger



DEAE-anion exchanger

CEX Proven Functionalities

Bead	Functionality	Page
Nuvia™ Macroporous hydrophilic acrylamido polymer Robust chemical stability Particle size range, 50–85 µm	**Nuvia S** High-capacity capture High salt tolerance Nonaffinity-based platform purifications	10
	Nuvia HR-S High-resolution polish Aggregate removal Isoform separation Glycoform separation	14
UNOsphere™ One-step polymerization Macroporous hydrophilic polymer High porosity Fast mass transfer Particle size range, 80–120 µm	**UNOsphere S** Unique selectivity Isoform separation Viral clearance	28
Macro-Prep® Polymethacrylate bead matrix High ligand density Macroporous average pore radius, 60–90 nm	**Macro-Prep High S** Unique selectivity Flow-through purifications Cost effective	42
	Macro-Prep CM Unique selectivity Weak cation exchanger Cost effective	45
Specialty Styrene divinylbenzene Strongly basic cation exchangers Separation of compounds <3,000 daltons	**AG® 50** Ultrapurification of buffers and ionic reagents Sample preparation Metal separations Weak acid separations Peptide separations Amino acid separations Nucleotide separations	70
Specialty Styrene divinylbenzene Strongly basic cation and anion exchangers Separation of compounds <3,000 daltons	**AG 501** Deionizition of water, urea, acrylamide, formamide, glyoxal, and PEG Water softening Waste treatment in nuclear power plants to remove radioactive substances from water Condensate polishing Ultrapurification of buffers and ionic reagents	80
Specialty Styrene divinylbenzene Weak cation exchange Contains paired iminodiacetate ions	**Chelex® 100** Removal of trace metals Concentration of glyphosate Removal of metals from buffers	89

AEX Proven Functionalities

Bead	Functionality	Page
Nuvia Macroporous hydrophilic acrylamido polymer Robust chemical stability Particle size range, 50–85 µm	**Nuvia Q** High capacity Selectivity DNA removal Viral clearance Endotoxin clearance	18
UNOsphere One-step polymerization Macroporous hydrophilic polymer High porosity Fast mass transfer Particle size range, 80–120 µm	**UNOsphere Q** DNA removal Viral clearance	31
Macro-Prep Polymethacrylate bead matrix High ligand density Macroporous average pore radius, 60–90 nm	**Macro-Prep High Q/ High Q-3HT** Unique selectivity Blood fractionation Large biomolecule purification Cost effective	47
	Macro-Prep DEAE Unique selectivity Weak anion exchanger Cost effective	50
Specialty Styrene divinylbenzene Strongly basic anion exchangers Separation of compounds <3,000 daltons	**AG 1/AG 2** Ultrapurification of buffers and ionic reagents Metal separations Peptide separations Amino acid separations Nucleotide separations Nitrate removal Purification of sugar compounds Fermenation applications	75
Specialty Styrene divinylbenzene Strongly basic cation and anion exchangers Separation of compounds <3,000 daltons	**AG 501** Deionization of water, urea, acrylamide, formamide, glyoxal, and PEG Water softening Waste treatment in nuclear power plants to remove radioactive substances from water Condensate polishing Ultrapurification of buffers and ionic reagents	80

IEX resins can be broadly divided into two types — cation and anion — depending upon the class of charged species they are capable of binding. CEX resins contain anionic (negatively charged) ligands and hence bind cationic (positively charged) species; for AEX resins, the reverse is true. Within each class there are primarily two ligand types that are commercially available: for CEX resins, the ligands contain sulfonyl (S) or carboxyl (CM) groups; for AEX resins, quaternary ammonium (Q) or diethylaminoethyl (DEAE) functionalities. Variants of these four basic ligands also exist (for example, phosphate-based CEX resins).

The choice of which resin to use at a particular step in a purification process is dictated by not only the character of the feedstream but also the desired outcome. For example, most material coming from cell culture contains large amounts of nucleic acids owing to cell death during fermentation or harvest collection. This large number of negatively charged nucleic acid species will bind strongly to AEX resins, resulting in reduced binding capacity for the target biomolecule; in addition, these species could be difficult to remove during resin cleaning. Accordingly, CEX is preferred for a capture step because the bulk of the nucleic acids will generally flow through the column during loading.

As the purification process progresses, often the pIs of the target protein and remaining impurities can dictate the type of resin to use, or at least how it will be used. If, for example, a basic protein has a number of impurities with similarly high pIs, then separation on a CEX resin may be possible, whereas all of the species would simply flow through AEX resin types. Impurities with an opposite charge from the target protein can be separated by either type of resin; for example, an acidic target protein with basic impurities can be polished by either binding the target protein to an AEX resin, allowing the negatively charged impurities to flow through; or by passing the feedstream through a CEX resin, allowing the target protein to flow through while retaining the impurities. These concepts are not always applicable, since a protein with an overall positive charge may nevertheless possess patches of negative charge that would allow it to bind to both anion and cation exchange resins at the same pH.

Once the charge type of the resin has been determined, the next step is to establish exactly which ligand will work best. No hard and fast rules exist to answer this question; often, for example, performance of a DEAE-based resin will be superior to that of a Q-type resin. Much depends on the exact molecular surface interaction between the ligand and the protein of interest. All else being equal, Q- or S-type resins should be selected based on the amount of buffer or buffering species necessary for equilibrating and operating the column.

Another characteristic is the desired capacity of the resin. The higher the binding capacity, the less resin and buffer needed for processing. This variable is more important for high-dose products such as antibodies than it may be for biomolecules that are highly active in minute quantities. Binding capacity may also be more important earlier in the process, where the total capacity will be affected in part by potentially large quantities of impurities.

Finally, the base matrix itself plays a role because biomolecules interact with the matrix as well as the ligand. Macro-Prep Resin is a relatively hydrophobic matrix, whereas UNOsphere and Nuvia Resins are much more hydrophilic.

MIXED-MODE

FUNCTIONAL GROUPS / APPLICATIONS

Mixed-Mode Chromatography

Mixed-mode chromatography depends on more than one interaction principle occurring between stationary phase and solute. Table 1 lists the various interaction principles and their application to process-scale resins.

Table 1. Chromatography mode interaction principles.

Mode	Principle	Nuvia™ cPrime™ Mixed-Mode Resins	Ceramic Apatites
CEX	Electrostatic binding	X	X
AEX	Electrostatic binding		
HIC	Hydrophobic complex formation	X	
C-sites	Metal chelate formation		X
Hydrogen bonding	Hydrogen bonding	X	
Phosphate-specific interactions			X

Mixed-Mode Proven Functionalities

Bead	Functionality	Page
Nuvia Macroporous hydrophilic acrylamido polymer Robust chemical stability Particle size range, 50–85 µm	**Nuvia cPrime** Unique selectivity Nonaffinity capture Salt tolerant mAb L chain fragment removal Aggregate removal	**21**
Specialty Styrene divinylbenzene Strongly basic cation and anion exchangers Separation of compounds <3,000 daltons	**AG® 501** Deionization of water, urea, acrylamide, formamide, glyoxal, and PEG Condensate polishing Ultrapurification of buffers and ionic reagents	**80**
Ceramic Hydroxyapatite Nonpolymer-based material Rigid high purity hydroxyapatite Controlled pore and particle sizes Particle sizes: 40 and 80 µm	**CHT™/MPC™** Highest clearance: aggregate removal Dimer removal Retroviral clearance DNA clearance Endotoxin removal	**58**
Ceramic Fluoroapatite Nonpolymer-based material Rigid high purity hydroxyapatite Controlled pore and particle sizes Particle sizes: 40 µm Acidic protein separation requiring pH as low as 5.6	**CFT™** Highest clearance: aggregate removal Dimer removal Retroviral clearance DNA clearance Endotoxin removal	**64**

Design Principles of Nuvia cPrime

Mixed-mode resins are generally designed with the addition of a spacer and ligand to a bead matrix. The ligand design impacts the strength and specificity of the interactions. Figure 1 depicts the chemical structure of the Nuvia cPrime ligand. Multiple interaction permutations are provided by the ligand and biomolecule (Figure 2).

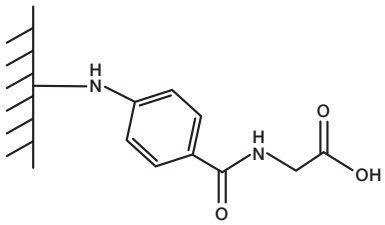


Fig. 1. Nuvia cPrime ligand.

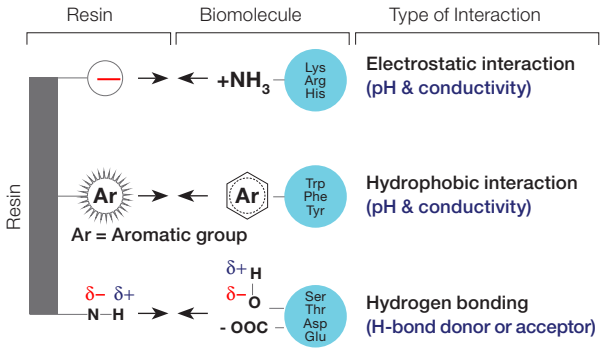


Fig. 2. Interaction permutations of the ligand and biomolecule.

Design Principles of Ceramic Apatites

CHT Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is unique as the base matrix itself provides the multiple modes of interaction (Figure 3).

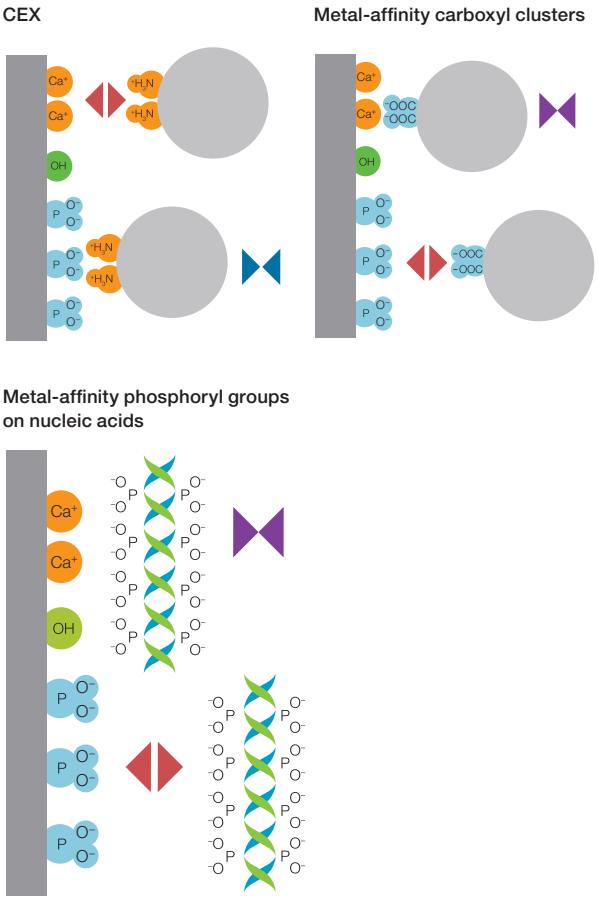


Fig. 3. Schematic representation of CHT binding mechanism. Biomolecule (●); metal affinity (⋈); electrostatic repulsion (⋈); electrostatic attraction (⋈).

Separation Principles

Multiple interactions between stationary and mobile phases can lead to unique selectivity and facilitate separation of closely related proteins and contaminants. Selectivity during binding, washing, and elution can be achieved by manipulating buffer components, conductivity, and/or pH.

Modulation of each interaction type must consider buffer effects on both the resin structure and protein. For example, the charge on the (typically weak) IEX groups on the solid support can be changed from neutral to positive (for amino-based ligands) or to negative (for carboxyl-based ligands) by varying the pH of the buffer. In addition, the charge on those amino acids, which specifically bind to the IEX functionalities, can be similarly changed. In a case where the charge on the protein and ligand are opposite, binding is enhanced. When the ligand and protein share the same charge, elution is enhanced.

Similarly, in a situation where both ligand and the protein binding site are neutral, hydrophobic interactions are enhanced. Generally, when both entities are of opposite charge, hydrophobic interactions near the charged amino acids are promoted. The opposite is true when both sites have the same charge.

Varying the salt concentration generally has opposite effects on ionic and hydrophobic interactions. Increasing the salt concentration reduces ionic interactions but promotes hydrophobic interactions; this is the basis for the relatively constant binding capacity of mixed-mode resins over a fairly broad conductivity range.

Finally, adding a variety of mobile-phase modifiers can also affect the interactions (Table 2).

Table 2. Effect of modifiers on interactions between biomolecules and resins.

Modifier	Modulated interaction
Arginine	Electrostatic ↓ Hydrophobic ↓ H-bonding ↓
Guanidine-HCl	Electrostatic ↓ Hydrophobic ↓ H-bonding ↓
Urea	Hydrophobic ↓ H-bonding ↓
Propylene glycol	Electrostatic ↑ Hydrophobic ↓
Glycine	Electrostatic ↓ Hydrophobic ↑

↓ interaction is weakened
↑ interaction is strengthened

Advantages

A variety of advantages of mixed-mode interactions have been observed beyond simply finer discrimination between product and impurities. They include:

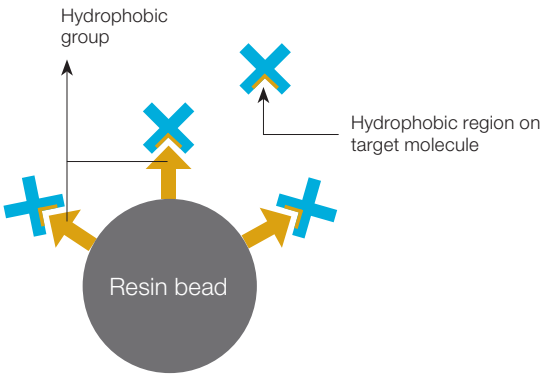
- Improved yield and activity via advantageous use of charge-charge repulsion between ligand and protein (Simmonds and Yon 1977)
- Improved design space for protein binding (Burton et al. 1997; Hamilton et al. 2000)
- Minimal feed manipulation prior to binding
- Mild condition to preserve activity

HYDROPHOBIC INTERACTION

FUNCTIONAL GROUPS / APPLICATIONS

Hydrophobic Interaction Chromatography

HIC involves the separation of protein molecules based on a differential interaction of these molecules with hydrophobic sites on the surface of a solid support. In the separation process, hydrophobic patches on the protein surface interact with hydrophobic molecules immobilized on a hydrophilic solid phase surface. Hydrophobic interactions are driven by the extrusion of a monomolecular layer of ordered water molecules covering two adjacent hydrophobic surfaces into less ordered bulk water with a concomitant increase in entropy. This entropy-driven interaction between nonpolar groups in water is the basis for HIC. Hydrophobic interactions on the protein surface are due primarily to amino acids containing aromatic (tyrosine, tryptophan) or alkyl (leucine, valine, isoleucine) side chains.



HIC Proven Functionalities

Bead	Functionality	Page
Macro-Prep® Polymethacrylate bead matrix High ligand density Macroporous average pore radius, 60–90 nm	**Macro-Prep Methyl and t-Butyl** Strong and weak hydrophobic regions Enyzme purification Cost effective	**54**

Functional Properties

Binding and elution of proteins from HIC resins is, in some sense, the opposite of these interactions with IEX ligands. HIC interactions are strengthened by increasing salt concentration, often using ammonium sulfate. Desorption is achieved by lowering the salt concentration and/or adding mobile phase modifiers such as alcohols or surfactants.

There are two basic types of HIC resins. The first contain aromatic ligands, such as a phenyl group. The second contains alkyl ligands and can be either straight-chain or branched. The interactions between a given protein and HIC resins are not necessarily predictable, and it is best to try members of both resin classes.

HIC resins are often used because they provide an orthogonal purification technique to IEX or affinity chromatography. In addition, many proteins, when they denature or are incorrectly folded, expose hydrophobic amino acids that are otherwise not normally accessible. Therefore, HIC resins can be used to remove these species from a process feedstream. HIC is often used to remove aggregated target proteins during a purification process.

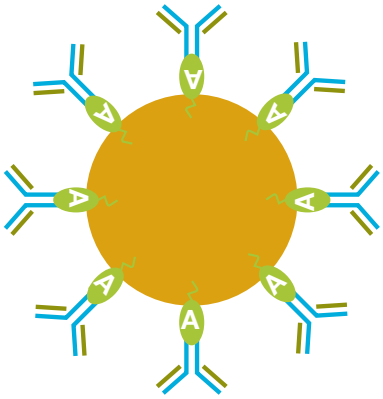
Because HIC requires high salt concentrations for binding, this step is frequently employed following ammonium sulfate precipitation or elution from IEX resins. HIC is typically the second or third step in a purification workflow. HIC resins are rarely used in the capture step due to their relatively low binding capacity and also because the large volumes coming from fermentations would require unacceptably large amounts of salt for the binding step.

AFFINITY

FUNCTIONAL GROUPS / APPLICATIONS

Affinity Chromatography

Affinity chromatography, as its name implies, relies on the ability of ligands to bind to specific well-defined sites on proteins. The most commonly used affinity ligand is Protein A, which binds to many subclasses of γ-immunoglobulins (IgGs). The binding interaction is primarily in the Fc domain; however, interactions with the Fab region have also been documented. Binding is typically performed directly from fermentation or other source materials and high yields and purities can be expected.



Affinity Proven Functionalities

Bead	Functionality	Page
UNOsphere™ One-step polymerization Macroporous hydrophilic polymer High porosity Fast mass transfer Particle size range, 80–120 µm	**UNOsphere SUPrA™** Fc fusion protein purification Mild elution conditions Low elution volumes	**35**

Functional Properties

Although all Protein A ligands work in the same basic manner, there are many engineered versions available. In some cases, a truncation of the coding gene has led to variations with reduced Fab binding. In others, site-specific mutations have produced Protein A variants with increased base stability for improved cleaning and sanitization.

Another commonly used class of affinity ligands is that employed in IMAC. These ligands were designed to specifically bind transition-state metals. In turn, the bound metals interact with histidine, cysteine, and phosphate groups on the surface of proteins. IMAC resins are perhaps best known as specific ligands for histidine-tagged proteins; however, a polyhistidine sequence is not necessary for good binding. The choice of metal ion to be immobilized on the IMAC ligand depends on the application. While trivalent cations such as Al^{3+}, Ga^{3+}, and Fe^{3+} or tetravalent Zr^{4+} are preferred for capture of phosphoproteins and phosphopeptides, divalent Cu^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+} ions are used for purification of histidine-tagged proteins and proteins with other presentations of surface histidine and/or cysteine.

IMAC ligands that bind in different ways to metal ions have been produced. In some cases, the ligands are tetradentate, meaning that they coordinate with the ligand via four valencies, leaving two valencies on the metal to interact with two protein residues. Other ligands are pentadentate, leaving one valency available for protein binding. As one might expect, combinations of different metals and ligands can produce supports with different affinities for individual proteins and mixtures.

Advantages

The main benefit of affinity resins is their high degree of specificity coupled with high recoveries. Protein A resins almost always deliver yields of at least 80% with between 2 and 5 log clearance of host cell proteins, DNA, and other impurities. IMAC resins can be used for the purification of histidine-tagged proteins as their binding affinity is high enough that proteins with noncontiguous histidine sequences are eliminated during a wash step. For other uses, IMAC resins are often employed as intermediate polishing steps in orthogonal approaches that could also include IEX and HIC.

Because of their high affinity, Protein A and IMAC resins (when used for histidine-tagged proteins) are often used for capture, with step elutions rather than gradients, for protein recovery. For non–histidine-tagged proteins, IMAC resins provide a solid orthogonal intermediate or final polishing in a purification process.

SPECIALTY

FUNCTIONAL GROUPS / APPLICATIONS

Specialty Chromatography

In addition to the more common ligand/support combinations for protein purification, a variety of specialty resins are available for specific tasks. The specialty resins described here all have pore sizes with exclusion limits of 2,700 daltons unless otherwise noted. As such, they are suitable for the purification/separation of small compounds but not larger species such as proteins.

Speciality Proven Functionalities

Bead	Functionality	Page
Specialty Styrene divinylbenzene Strongly basic cation exchangers Separation of compounds <3,000 daltons	**AG® 50** Ultrapurification of buffers and ionic reagents Sample preparation Metal separations Weak acid separations Peptide separations Amino acid separations Nucleotide separations	70
Specialty Styrene divinylbenzene Strongly basic anion exchangers Separation of compounds <3,000 daltons	**AG 1/AG 2** Ultrapurification of buffers and ionic reagents Metal separations Peptide separations Amino acid separations Nucleotide separations Nitrate removal Purification of sugar compounds Fermentation applications	75
Specialty Styrene divinylbenzene Strongly basic cation exchangers Separation of compounds <3,000 daltons	**AG 501** Deionization of water, urea, acrylamide, formamide, glyoxal, and PEG Condensate polishing Ultrapurification of buffers and ionic reagents	80
Specialty Molecular weight exclusion <2,000 daltons Polymeric beads Compatible with solvents (for example, alcohols) No expansion or contraction of beads	**Bio-Beads™ SM-2** Absorption of nonpolar substances Removal of detergents such as Triton X-200 Removal of organics Cleanup of drugs from plasma and urine Extraction of dyes and mycotoxins from food products	83
Specialty Porous styrene divenlybenzene Exclusion limit range, 400–14,000 daltons Compatible with solvents (for example, alcohols) No expansion or contraction of beads	**Bio-Beads S-X** Quantification of pesticides and rodenticides Measurement of organic priority pollutants in sludge Separation of polycyclic aromatic compounds Assessment of tissue reaction to biomaterial Fractionation of halogenated environmental contaminants Separation of tall oil components, lipids, alkalines, 21 fatty acids, hydrocarbons, and polystyrenes	86
Specialty Styrene divinylbenzene Weak cation exchange Contains paired iminodiacetate ions	**Chelex® 100** Ultra purification of buffers and ionic reagents Glyphosate concentrations SDS removal Deionization of urea, formamide, glyoxal, acrylamide Sample preparations Metal separations Peptide separations Amino acid separations Nucleotide separations Nitrate removal Purification of sugar compounds	89

AG Resins comprise a set of IEX products based on a variably crosslinked styrene divinylbenzene copolymer lattice. Exclusion limits for this series range from ~400 to ~2,700 daltons. The resin types have either one ligand (anion or cation exchange) or a mixture of cation and anion exchange functionalities. In all cases, the ligands are either strongly basic or strongly acidic.

Bio-Beads SM-2 is a nonpolar polystyrene adsorbent composed of analytical-grade, neutral, macroporous polymeric beads for use in HIC. These beads can be used in aqueous solution and with solvents including alcohols, petroleum ether, diethyl ether, and hexane, or with solvent mixtures, without expansion or contraction of the beads.

Chelex 100 is a styrene divinylbenzene resin containing paired iminodiacetate ions, which act as chelating groups in binding polyvalent metal ions. Chelex 100 is a weakly acidic CEX resin due to its carboxylic acid groups, but it differs from ordinary ion exchangers because of its high selectivity for metal ions and its much higher bond strengths.

Types Available

AG 1 Resins contain strongly basic (quaternary amine) ligands. AG 50 Resins have a strongly acidic (sulfopropyl) functionality. AG 501 Resins combine cation and anion exchange functionalities. All of these are derived from styrene divinylbenzene copolymer.

Benefits

The lower crosslinked AG Resins, particularly the 2% crosslinked AG 1-X2 Resins, are useful for the sorption and fractionation of relatively high molecular weight substances such as peptides, ribo- and deoxyribonucleotides, and uranium. The higher crosslinked resins, particularly the 8% crosslinked AG 1-X8 Resins, are used for sorption, exchange, and separation of low molecular weight inorganic anions and in applications such as cyclic nucleotide assays and fractionation of organic acids. The AG 50 Resins are used for correspondingly sized cationic species. The AG 2 Resins follow a similar profile as AG 1 but are used for positively charged species.

AG 501, containing both anionic and cationic groups, is used for the deionization of various solutions such as urea.

Bio-Beads SM-2 Resins are frequently used in the biopharmaceutical industry for the removal of surfactants from feedstreams, for example following a surfactant solubilization step. The resin is also used in the purification, separation, and identification of a wide variety of compounds, including water-soluble steroids, phenols, drugs, pesticides, and trace organics.

Bio-Beads S-X Resins are used with organic solvents for the separation of small molecules such as polycyclic aromatics, tall oil components, lipids, and fatty acids.

Key Applications

The main uses for each of these resins are described in the Table 1.

Table 1. Uses for various beads in specialty chromatography.

Resin	Matrix	Ligand	Main Uses
AG 1	SDVB	Strongly basic	Removal, exchange, and purification of small anionic species
AG 50	SDVB	Strongly acidic	Removal, exchange, and purification of small cationic species
AG 501	SDVB	Mixed strongly acidic and basic	Deionization of nonionic solutions (for example, urea)
Chelex 100	SDVB	Chelating	Removal of transition-state metals, buffer purification, and removal of PCR inhibitors
Bio-Beads SM-2	SDVB	None	Removal of surfactants
Bio-Beads S-X	SDVB	None	Gel filtration of nonpolar molecules (for example, pesticides and herbicides), sample cleanup, and separation of lipids

Notes

CHAPTER 3

Case Studies

CASE STUDIES

Monoclonal Antibody Purification Using UNOsphere™ Resins and CHT™ Ceramic Hydroxyapatite

Background

The first step in purification of an important class of therapeutic proteins such as mAbs is their capture from plasma or tissue culture supernatants. Protein A–based resins are by far the most common class of affinity products used for this purpose. They bind with high affinity to the Fc region of most subclasses of antibodies and are one of the standard tools used in antibody capture and purification.

UNOsphere SUPrA™ Resin can be integrated in a workflow of mAb purification in combination with UNOsphere Q Resin and CHT Ceramic Hydroxyapatite to effectively purify mAbs. The results demonstrate that UNOsphere SUPrA, in combination with the subsequent polishing steps, delivers a highly pure mAb.

Method

Two antibodies were purified using a workflow consisting of three steps: capturing the antibodies with UNOsphere SUPrA Resin, intermediate polishing with UNOsphere Q IEX Resin, and final polishing using CHT Ceramic Hydroxyapatite.

Capture with UNOsphere SUPrA Resin

Column: 1 ml, 0.56 x 4 cm

Step	Buffer	CV	Flow Rate, cm/hr
Equilibration	1x PBS	10	300
Sample Loading	CHO cell culture supernatent	–	300
Wash	1x PBS	20	300
Elution	100 mM glycine, pH 3.0	–	300

Pooled mAb fractions were incubated for 1 hr at pH 3.0 (to mimic a virus deactivation step) and then adjusted to pH 7.8 using 10 mM sodium phosphate, pH 8.9.

Intermediate Polishing with UNOsphere Q Resin

Column: 1 ml, 0.56 x 4 cm

Step	Buffer	CV	Flow Rate, cm/hr
Equilibration	10 mM sodium phosphate, pH 7.8 (buffer A)	10	300
Sample Loading	UNOsphere SUPrA eluate adjusted to pH 7.8	–	300
	Buffer A	10	300
Strip/Sanitization	1 N NaOH	10	300

The mAb in the flowthrough was collected and the pooled fractions adjusted to pH 6.8 using 20 mM sodium phosphate, pH 4.0.

Final Polishing with CHT Type I, 40 µm

Column: 1 ml, 0.56 x 4 cm

Step	Buffer	CV	Flow Rate, cm/hr
Equilibration	10 mM sodium phosphate, pH 6.8 (buffer A)	10	300
Sample Loading	Pooled fractions from UNOsphere Q Column adjusted to pH 6.8	–	300
Wash	Buffer A	15	300
Elution	Salt gradient formed between buffer A and 10 mM sodium phosphate, 1 M NaCl, pH 6.8 (buffer B)	25	300
Strip	500 mM sodium phosphate, pH 6.5 (buffer C)	15	300

Results

Although there were substantial levels of aggregates in mAb2 eluted from the capturing step (Figure 1A) the final CHT Ceramic Hydroxyapatite pool appears to be completely devoid of these unwanted materials (Figure 1B). Interestingly, one consistent feature of combining CHT Ceramic Hydroxyapatite chromatography with Protein A capture is the remarkable ability to clear these higher-order structures.

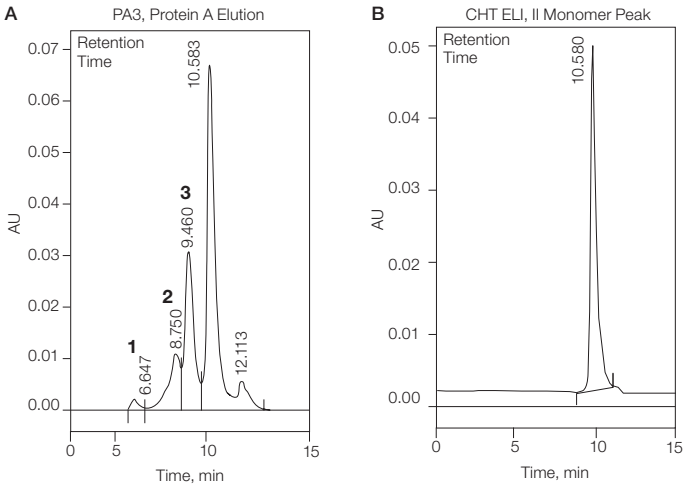


Fig. 1. Removal of impurities by CHT chromatography. Elution fractions from the capture (**A**) and final polishing (**B**) chromatographic steps were analyzed by HPLC-SEC using a Bio-Sil® SEC 400-5 to evaluate levels of contamination. The peaks corresponding to aggregates (peaks 1 and 2) and dimers (peak 3) are removed by CHT Ceramic Hydroxyapatite to yield a clean sample.

UNOsphere SUPrA Resins show an excellent ability to clear contaminating host cell proteins and DNA. CHT Ceramic Hydroxyapatite was capable of clearing Protein A to the level of detection in all cases (Table 1).

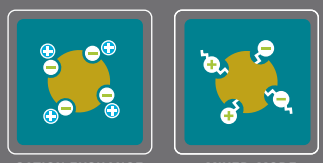
Table 1. mAb1 and mAb2 impurity clearance data.

	Protein A, ng/mg		HCP, ng/mg		DNA, ng/mg		Aggregate/Dimer Content, %	
Sample	**mAb1**	**mAb2**	**mAb1**	**mAb2**	**mAb1**	**mAb2**	**mAb1**	**mAb2***
Cell culture supernatant	–	–	3.5 x 10	1.4 x 10^6	>5 x 10^3	>1.6 x 10^5	ND	ND
UNOsphere SUPrA fraction	3	ND	175	197	18.6	19	ND	42
UNOsphere Q fraction	0.8	112	<3	86	3.5	1.9	ND	40
CHT ceramic hydroxyapatite fraction	<0.7	<0.4	<2	48	2.5	3	<0.03/<0.03	<0.1

–, Not applicable. ND, not determined.
* Dimer content was not determined for mAb2.

Summary

UNOsphere SUPrA Affinity Resin can be used to remove impurities to extremely low levels. In a workflow comprising a capture step and two polishing steps, UNOsphere SUPrA, UNOsphere Q, and CHT Ceramic Hydroxyapatite were able to provide mAbs of high quality and purity.



CASE STUDIES

A Purification Strategy for Clinical-Grade Monoclonal Antibody Using Hydrophobic CEX Chromatography

Background

mAbs are currently the most important class of therapeutic proteins. Advances in upstream process technologies have led to tremendous improvement of mAb titers in mammalian cell culture. Increases in fermentation volume and the protein mass produced have made the timely processing of harvested material extremely challenging. This is further compounded by the elevated levels of process- and/or product-related impurities resulting from prolonged fermentation and substantially higher cell density in the expression culture. Chromatographic resins with high capacity and improved chromatographic and operational performance offer the latest productivity tools to address downstream process challenges. We have employed two next generation ultra high capacity ion exchangers, Nuvia™ S and Nuvia Q Resins, and Nuvia™ cPrime™ Hydrophobic CEX Resin, to effectively purify a mAb from CHO cell culture harvest. Our results demonstrate that this three-step nonaffinity workflow can effectively deliver highly purified mAbs with minimal feed conditioning.

Method

Capture on Nuvia S Resin

Column: 1 ml, 0.56 x 4 cm

Step	Buffer	CV	Flow Rate, cm/hr
Equilibration	20 mM sodium acetate, 20 mM NaCl, pH 4.7 (buffer A)	15	300
Sample Loading	CHO cell culture supernatent diluted 1:4 with dH_2O; adjusted to pH 4.7 with 1 M phosphoric acid; clarified with 0.2 µm filter	85	300
Wash	Buffer A	15	300
Elution	20 mM sodium acetate, 200 mM NaCl, pH 4.9 (buffer B)	15	300
Sanitization	1 N NaOH	5	300

Eluate was adjusted to pH 7.0 with 1 N NaOH.

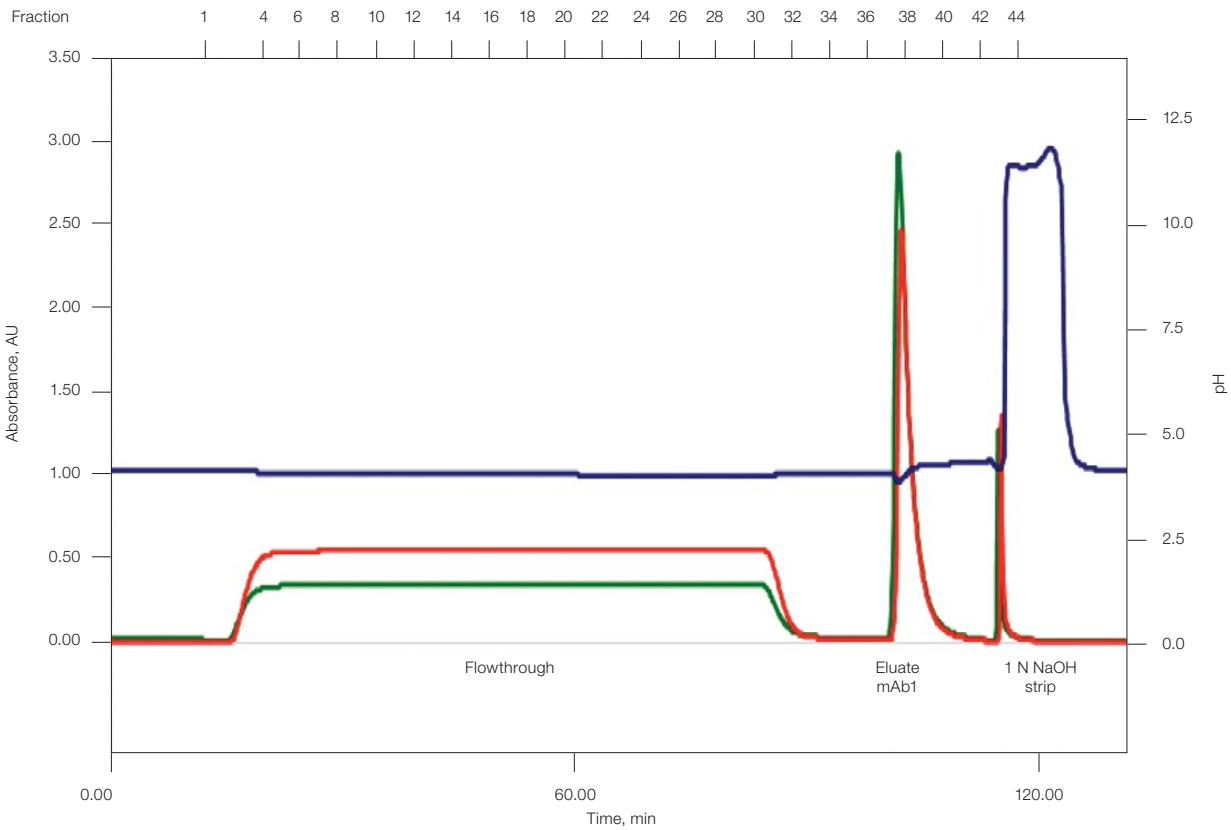


Fig. 1. Capturing mAb1 from CHO cell culture harvest using Nuvia S Resin. mAb1 eluted from the column (fractions 38–40) was subjected to further purification and purity analysis. OD 280 (—); OD 260 (—); pH (—).

Intermediate Polishing on Nuvia Q Resin

Column: 1 ml, 0.56 x 4 cm

Step	Buffer	CV	Flow Rate, cm/hr
Equilibration	10 mM sodium phosphate, 10 mM NaCl, pH 7.0 (buffer A)	10	300
Sample Loading	Eluate from Nuvia S Column adjusted to pH 7.0	10	300
Wash/Flowthrough	Buffer A	25	300
Strip	100 mM sodium phosphate, 1.5 M NaCl, pH 7.2 (buffer B)	10	300
Sanitization	1 N NaOH	5	300

The mAb in the flowthrough was collected and the pooled fractions were adjusted to pH 5.0 with 100 mM sodium acetate, 500 mM NaCl, pH 4.5.

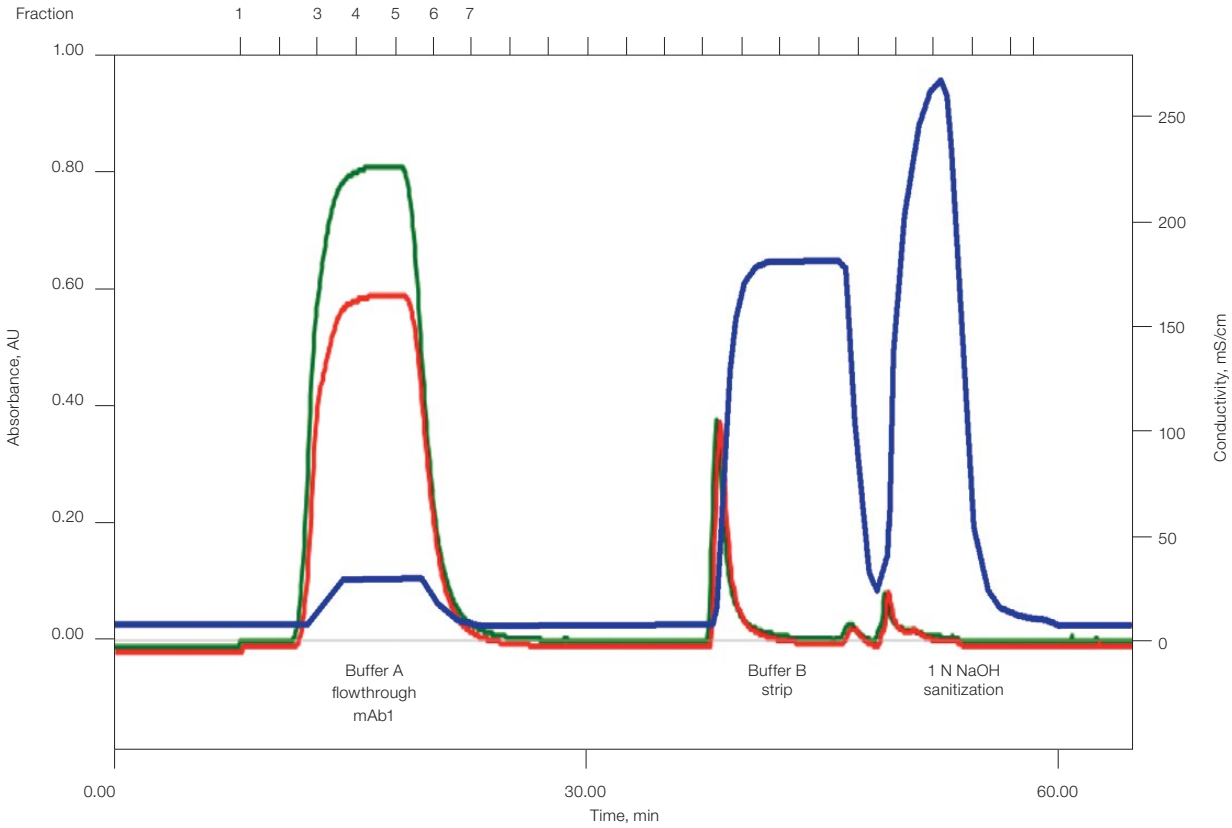


Fig. 2. Intermediate polishing purification of mAb1 in flow-through mode using Nuvia Q Resin. mAb1 in column flowthrough (fractions 3–6) was subjected to further purification and purity analysis. OD 280 (—); OD 260 (—); conductivity (—).

Final Polishing on Nuvia cPrime Resin

Column: 1 ml, 0.56 x 4 cm

Step	Buffer	CV	Flow Rate, cm/hr
Equilibration	50 mM sodium acetate, 125 mM NaCl, pH 5.0 (buffer A)	10	300
Sample Loading	Pooled fractions from Nuvia Q Column adjusted to pH 5.0	12	300
Wash	Buffer A	15	300
Elution 1	Salt gradient formed between buffer A and 50 mM sodium phosphate, 50 mM NaCl, pH 6.2 (buffer B)	15	300
Elution 2	Buffer B	5	300
Strip	200 mM sodium phosphate, 700 mM NaCl, pH 7.5	10	300
Sanitization	1 N NaOH	5	300

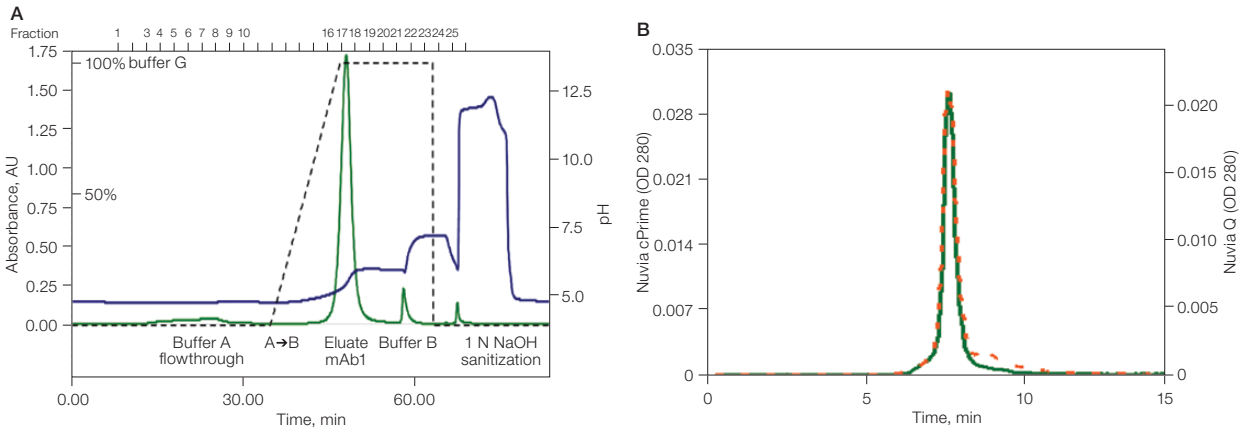


Fig. 3. Final polishing purification of mAb1 using Nuvia cPrime Resin. A, chromatogram of mAb1 purification on a Nuvia cPrime Column: OD 280 (—); pH (—); percentage of buffer G (- - -). mAb1 eluted from the column (fractions 17–19) was collected and subjected to purity analysis. **B**, HPLC-SEC comparison of pooled Nuvia Q fractions (- - -) and pooled Nuvia cPrime fractions (—).

Results

Nuvia S is a high-capacity CEX resin with readily available negatively charged groups. Meanwhile, host-cell DNA contaminants present in the clarified cell culture harvest are repelled by these ligands. Consequently, dsDNAs mostly presented in the flow-through fractions during the capture step, as revealed by the absorbance trace at 260 nm (Figure 1), resulting in a remarkable, more than 3-log reduction of the dsDNA contamination level (Table 1).

Table 1. Impurity clearance.

Sample	Host Cell Proteins, ng/mg	Host Cell dsDNAs, ng/mg	Aggregate Content, %
Cell culture supernatant	6.3×10^4	9.3×10^4	Not determined
Nuvia S fraction	2.6×10^3	17	Not determined
Nuvia Q fraction	59	4.1	Not determined
Nuvia cPrime fraction	5.5	Not detected (<0.008)	<0.9

Further clearance of host-cell DNAs and proteins was accomplished by intermediate polishing on the Nuvia Q Column. The primary concern for this purification step was to maximize recovery of the mAb in the flow-through fractions while binding the highest level of contaminants to the Nuvia Q Column. We eventually conducted a flow-through purification of mAb purification at pH 7.0, slightly below the mAb's determined pI value of 7.2 (Figure 2). Both protein and DNA contaminants were dramatically reduced under this optimized condition.

Nuvia cPrime is a hydrophobic CEX resin (mixed mode). Unlike traditional CEX resin, Nuvia cPrime is tolerant to salts in the feedstream because of its hydrophobic character. The partially purified mAb from the Nuvia Q polishing step could be loaded onto the Nuvia cPrime Column following a simple pH adjustment to positively charge the mAb without extensive dilution or buffer exchange for conductivity reduction. Such behavior makes Nuvia cPrime distinct from conventional hydrophobic interaction resins, which require high salt levels for effective protein binding. Under the selected condition, only the desired full-length mAb was retained by the column. The bound intact mAb was eluted by a buffer with slightly higher pH and lower conductivity, which suggests that the mAb was likely selectively bound by Nuvia cPrime via a combination of ionic and hydrophobic interactions. This chromatographic step was also very effective at eliminating host cell impurities (Table 1).

The final mAb preparation was essentially nucleic acid–free because nucleic acids were unretained due to electrostatic charge repulsion between the negative hydrophilic DNA and the negatively charged hydrophobic ligand of Nuvia cPrime. The purity of the mAb after this three-step purification was assessed by HPLC-SEC analysis (Figure 4).

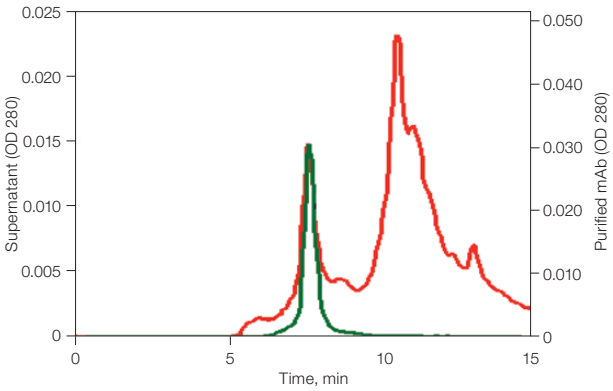


Fig. 4. Three-step purification of mAb1 using Nuvia cPrime Resin. HPLC-SEC comparison of cell culture supernatant (—) and purified mAb1 (—).

Nuvia cPrime Resin's higher affinity for the full-length mAb compared to process impurities and by-products, gives this final polishing step its powerful purification benefit. It can be thought of as an orthogonal approach, combining CEX and HIC in a single step to achieve unique selectivity for a target protein molecule. Both the binding and elution conditions used for this chromatographic step were effective yet gentle, which is crucial for maintaining the integrity of the monomeric mAb.

Summary

Two IEX resins, Nuvia S and Nuvia Q, were used in the first two steps to efficiently capture the target mAb from cell culture harvest and to effectively remove impurities produced during the fermentation process. The extraordinarily high binding capacities of these chromatography resins make it possible to process increasing volumes of high-titer feed with existing production facilities and minimal buffer consumption, easing the pressure on capital investment and process development timelines. In the final polishing step, the orthogonal interaction modes afforded by Nuvia cPrime Resin offer unique selectivity for the full-length monomeric mAb molecules. This resin is a powerful tool for the removal of both product-related impurities and host-cell contaminants. The versatility of these resins has allowed us to arrange the order of these three chromatography steps in a sequence that requires no buffer exchange and minimal handling. Transitions between process steps are easy and straightforward. This process is designed to shorten cycle time and reduce buffer consumption, thus improving overall productivity. All chromatography resins are base stable and mechanically resilient (bulletins 5987, 6129, and 6242). They can be operated at high flow rates with low backpressures, providing the productivity, robustness, and process economics demanded by today's downstream manufacture of biopharmaceuticals.

CATION EXCHANGE

CASE STUDIES

Nuvia™ HR-S: Resolving mAb Deamidation Charge Variants

Background

Protein deamidation at neutral or alkaline pH is of significant concern to manufacturers of biotherapeutic proteins. Deamidation at asparagine and glutamine residues has been shown to affect the immunogenicity, stability, and activity of these biotherapeutic proteins. In addition, these degradations also lead to molecular heterogeneity, which can present challenges to the manufacturing of a consistent protein product. The specific challenge is that the change in structure of the protein is minimal as the amide group is substituted with a carboxylate. Furthermore, the overall charge of the protein changes by only –1 per deamidation, making charge-based separations such as IEX potentially difficult. The goal of the present work was to determine if a high-resolution CEX resin, Nuvia HR-S, is capable of resolving proteins differing by one or a few deamidation events.

Method

Intermediate Polishing on Nuvia HR-S Resin

Column: 1 ml, 0.5 x 5.0 cm

Step	Buffer	CV	Flow Rate, cm/hr
Equilibration	10 mM sodium acetate, pH 4.5 (buffer A)	–	300
Sample Loading	Protein A eluate diluted 1:4 in buffer A	–	300
Wash 1	Buffer A	–	300
Wash 2	10 mM sodium citrate, 10 mM sodium phosphate, pH 5.0 (buffer B)	–	300
Elution	Gradient of buffer B to 10 mM sodium citrate, 10 mM sodium phosphate, pH 8.0	15	300

Results

An antibody with known surface asparagines and glutamines was first isolated by Protein A affinity chromatography and then further purified on Nuvia HR-S in a pH gradient. In the resulting chromatogram (Figure 1), the elution peak contains a prominent leading shoulder. The shoulder and peak were analyzed by SDS-PAGE electrophoresis and both were shown to contain antibody heavy and light chains (Figure 2). Fractions 7–13 contain the target mAb; fractions 8 and 11 were selected for further analysis by mass spectrometry (Figure 3). Analysis of the data indicated that the shoulder fragment had a higher mass by approximately 2.5 mass units. Since deamidation produces a mass change of approximately +1 units, this means that the light chain in the shoulder of the antibody peak resulted from two or three deamidation events.

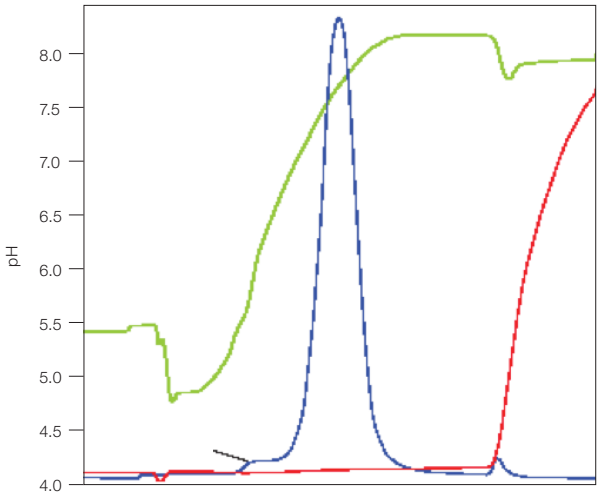


Fig. 1. Purification of the mAb on Nuvia HR-S. The distinct shoulder on the front side of the main peak is a modified mAb fragment. A_{280} (—); conductivity (—); pH (—).

Examination of the sequence in that region revealed the presence of two asparagine and three glutamine residues, consistent with the results (Figure 4).

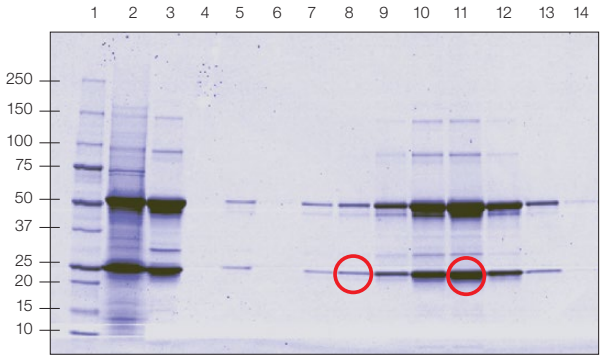


Fig. 2. SDS-PAGE analysis of chromatographic fractions. Lane 1, Precision Plus Protein™ Standards; 2, mAb cell culture harvest; 3, Protein A capture eluate. Lanes 4–14 show Nuvia HR-S Resin chromatography: 4–5, flowthrough 1 and 2; 6, wash; 7–14, elutions 1–8. The bands circled in red correspond to the bands used for mass spectrometry analysis in Figure 3.

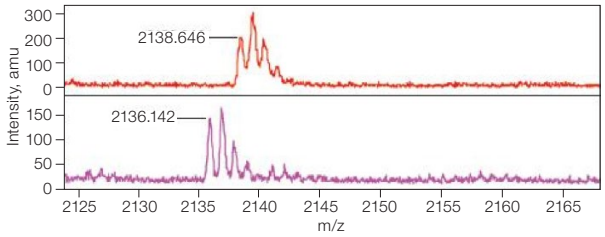


Fig. 3. Mass spectrometry analysis of the mAb. Analysis of the gel-purified proteins was carried out with an autoflex II MALDI-TOF Mass Spectrometer. Mass spectrometer analyses of the shouldered peak (top spectrum) and the main peak (lower spectrum) show a mass shift of about 2.5 amu between the two peaks, which is consistent with two or three deamidations on a single peptide. amu, atomic mass unit.

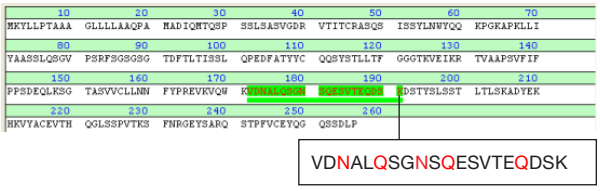
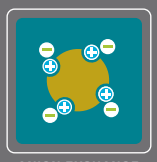


Fig. 4. Shoulder fraction protein sequence. Examination of the shoulder fraction protein sequence confirms deamidation events on a single mAb peptide.

Summary

The present study has shown that Nuvia HR-S Resin is capable of resolving antibodies differing by no more than two or three deamidation events. The ability to resolve such closely related species can significantly aid manufacturers in producing consistent biotherapeutic products.


ANION EXCHANGE


MIXED-MODE

CASE STUDIES

Development of a cGMP-Ready Purification Process for Adenovirus Purification

Background

Adenovirus vectors are effective tools for the transfer of genetic material into mammalian cells. They offer several advantages, including the capacity to accommodate up to 37 kb of foreign genetic material, very high infection efficiencies, the ability to infect a wide variety of both dividing and nondividing cell types, lack of integration into the host chromosome, and production systems capable of generating high virus titers. These and other qualities have led to adenoviruses being the most used gene transfer vectors in experimental therapies, accounting for 25% of all gene therapy trials; as of 2014, they had been used in almost 500 clinical trials.

Large-scale downstream processing of viruses for clinical applications poses challenges that arise, in part, from the viruses' large size and complexity. In the case of adenovirus, one intact virus particle (vp) contains over 2,700 protein subunits, has a mass of approximately 165 MDa, and has a diameter of approximately 0.1 µm. The complexity gives rise to thousands of charge variants, making it difficult to establish well-defined binding and elution conditions. Adenoviruses tend to be acid-labile, which further increases their complexity.

We developed a two-column cGMP-ready purification process for a recombinant adenovirus after screening five Bio-Rad chromatography resins. We show that the final process yields an active, concentrated virus product with purity, HCP levels, and DNA contamination comparable to clinical-grade products. The process is readily scalable and is sufficiently simple, rapid, and efficient to be considered for the production of clinical-grade viral vectors.

Method

Capture on Nuvia™ cPrime™ Resin

Column: 5 ml, 0.8 x 10 cm

Step	Buffer	CV	Flow Rate, cm/hr
Equilibration	25 mM histidine, pH 6.0 (buffer A)	10	120
Sample Loading	Culture supernatant diluted 1:3 with buffer A	48	120
Wash	Buffer A	5	120
Elution	75 mM Tris, 525 mM NaCl, pH 8.5	3	120

Polishing on Nuvia Q Resin

Column: 5 ml, 0.8 x 10 cm

Step	Buffer	CV	Flow Rate, cm/hr
Equilibration	75 mM, pH 8.0 (buffer A)	10	120
Sample Loading	Nuvia cPrime eluate diluted 1:1 with buffer A	2	120
Wash 1	75 mM, 250 mM NaCl, pH 8.0	2	120
Wash 2	15 mM Tris, 440 mM NaCl, pH 8.5	3	120
Elution	75 mM Tris, 1 M NaCl, pH 7.5	5	120

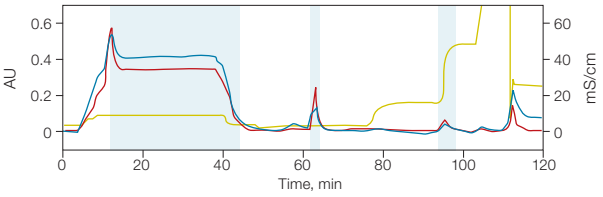
Results

Process Development

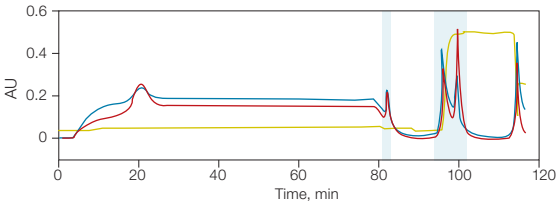
Mass Capture

As shown in Table 1, three resins had potential for use in a mass capture process — Nuvia cPrime, UNOsphere™ Q, and Nuvia Q. Of these, Nuvia cPrime was selected because the requirements for virus binding (low salt and low pH) made it poorly suited for use after an anion exchange capture operation (where the resulting feedstream would be expected to have high ionic strength and perhaps high pH).

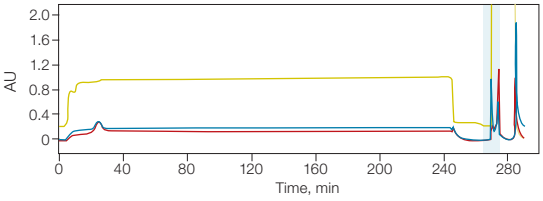
Developing the mass capture step using Nuvia cPrime involved replacing the buffer exchange step used in the screening study with a dilution step. Also, a nuclease digestion was introduced prior to column loading. Initial mass capture experiments were focused on reducing feedstream volumes and recovering virus. Example chromatograms, overlaid with the results of the transgene expression assay, illustrate the early progression of these experiments (Figure 1).



- Feed diluted 1:1 with 75 mM histidine, pH 6.0
- Wash = 75 mM histidine, pH 6.0
- Elution 1 = 75 mM Tris, pH 8.0
- Elution 2 = 75 mM Tris + 125 mM NaCl, pH 8.0



- Feed diluted 1:3 with 75 mM histidine, pH 6.0
- Wash = 75 mM histidine, pH 6.0
- Elution 1 = 75 mM Tris, pH 8.0
- Elution 2 = 75 mM Tris + 525 mM NaCl, pH 8.5



- Feed diluted 1:3 with 25 mM histidine, pH 6.0
- Wash = 25 mM histidine, pH 6.0
- Elution = 75 mM Tris + 525 mM NaCl, pH 8.5

Fig. 1. Iterative development of Nuvia cPrime capture. OD 260 (—); OD 280 (—); conductivity (—). Blue shading indicates detection of significant transgene expression.

Table 1. Results from the initial resin screening and their implications.

Column type	Virus in flowthrough/ wash	Virus in eluate	Notes/Implications
UNOsphere S (CEX)	+++	++	Poorly suited for virus purification in both bind-and-elute and flow-through modalities
Nuvia S (CEX)	+++	++	Poorly suited for virus purification in both bind-and-elute and flow-through modalities
Nuvia cPrime (MM)	—	++++	Partial elution in 125 mM NaCl, pH 6.5; hence, dilution of crude harvest required prior to column loading
UNOsphere Q (AEX)	—	++++	Could be considered for direct mass capture
Nuvia Q (AEX)	—	++++	Could be considered for direct mass capture

AEX Chromatography

Of the two AEX resins, UNOsphere Q and Nuvia Q, Nuvia Q was selected because it could adsorb virus at higher NaCl concentrations (Figure 2). It was therefore easier to work with downstream of the Nuvia cPrime capture step, where the eluate had an NaCl concentration of approximately 500 mM going on to AEX.

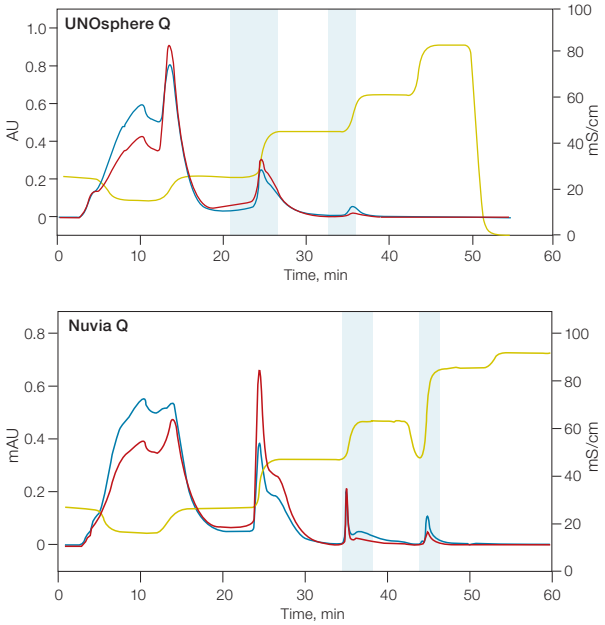


Fig. 2. Behavior of the crude harvest on the UNOsphere Q and Nuvia Q Columns. OD 260 (—); OD 280 (—); conductivity (—). Blue shading indicates detection of significant transgene expression.

AEX experiments with Nuvia Q were focused on attaining high product purity (Figure 3). In one late-stage experiment, a suspected HCP contaminant was prevented from eluting with the final product by increasing the pre-elution NaCl concentration.

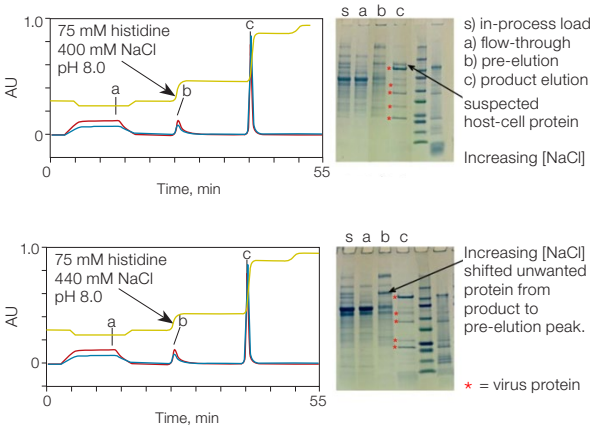


Fig. 3. Chromatograms and gels from AEX process development experiments. OD 260 (—); OD 280 (—); conductivity (—).
* Proteins presumed to be virus capsid components.

Final Process

Mixed-Mode Chromatography

Initial capture was accomplished using Nuvia cPrime Mixed-Mode Resin (Figure 4). This portion of the process achieved a tenfold reduction in processing volume and a significant reduction in feedstream contaminants (Figure 6, lanes 2–4).

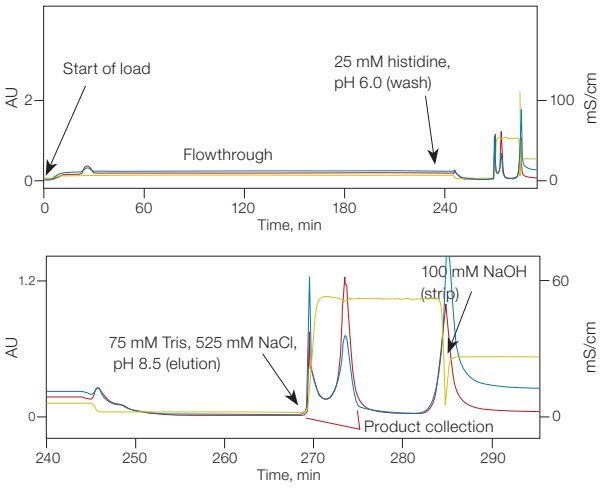


Fig. 4. Representative mixed-mode chromatogram. OD 260 (—); OD 280 (—); conductivity (—).

AEX Chromatography

Final virus purification was accomplished using Nuvia Q Resin (Figure 5). This portion of the process achieved an additional twofold reduction of product volume along with a significant improvement in product purity (Figure 6, lanes 4–7). Following this operation, nonvirus proteins were no longer evident by SDS-PAGE (Figure 6, lane 7).

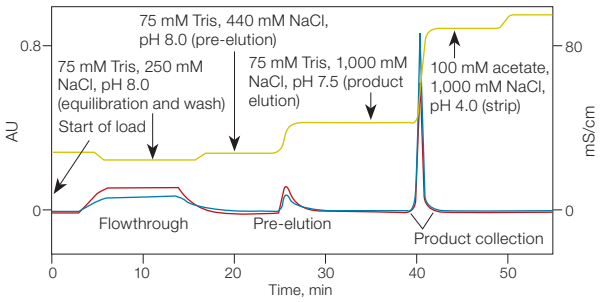
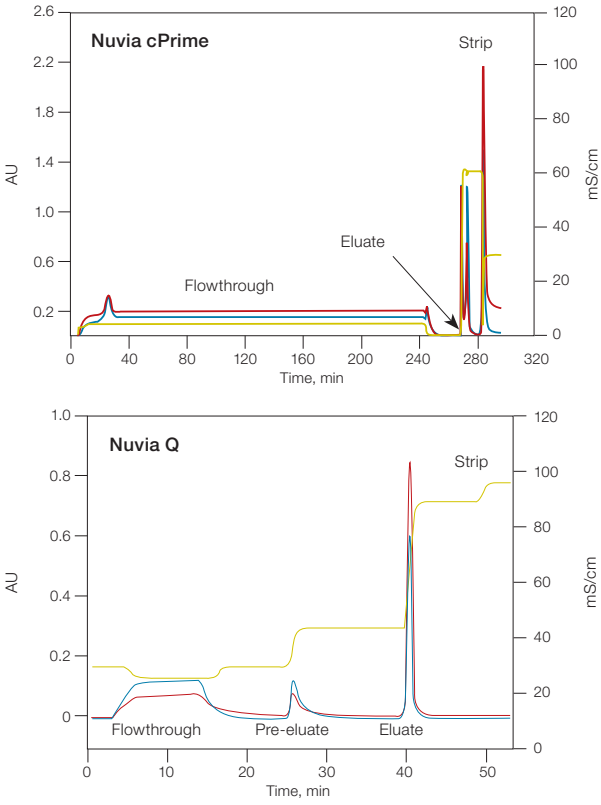


Fig. 5. Representative AEX chromatogram. OD 260 (—); OD 280 (—); conductivity (—).

Analysis of In-Process and Final Product

We used SDS-PAGE analysis to visualize the progressive reduction of contaminating proteins at each step of the purification process (Figure 6). The five most prominent viral proteins — hexon, penton, core (V), hexon (VI), and core (VII) — are readily visible in the final purified product (Figure 6, lane 7).



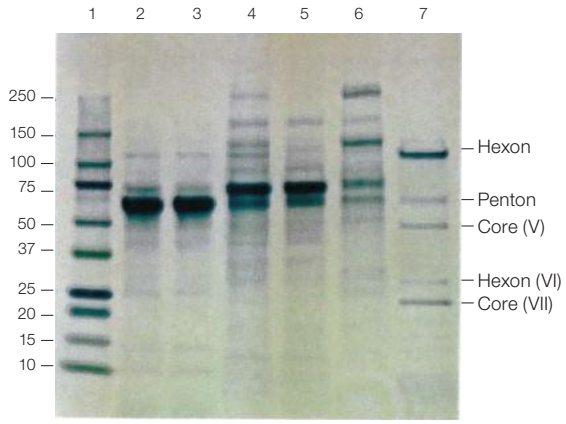


Fig. 6. SDS-PAGE of intermediates and the final product. Lane 1, MW marker; lane 2, Nuvia cPrime load; lane 3, Nuvia cPrime flowthrough; lane 4, Nuvia cPrime elution/Nuvia Q load; lane 5, Nuvia Q flowthrough; lane 6, Nuvia Q pre-elution; lane 7, Nuvia Q product. OD 260 (—); OD 280 (—); conductivity (—).

The data in Table 2 demonstrate an overall recovery of virus particles of approximately 54%, with DNA levels below detection and host cell protein at 2 ng/10^{10} particles. These values are well within current guidelines for clinical and perhaps commercial use.

Table 2. Viral particle recovery and impurity clearance.

Sample	Total virus ($x10^{11}$ particles)	Impurity levels (ng/10^{10} particles)	
		DNA	HCP
Bulk harvest	30.6	3,144	ND
Nuclease-treated harvest	31.8	30	3,022
Nuvia cPrime eluate	18.4	ND	58
Nuvia Q eluate	16.4	<0.02	2

ND, not determined.

Summary

The final process yields an active, concentrated virus product with purity, HCP, and DNA levels comparable to clinical-grade products. While the purification methods presented here were developed using the Ad5-E1+GFP model virus, they are expected to be applicable to recombinant adenoviruses in general, and to constructs derived from serotype 5 viruses in particular. The process is readily scalable and uses procedures and reagents compatible with cGMPs. Also, it is sufficiently simple, rapid, and efficient to be used for the production of clinical-grade virus-based gene therapy products and vaccines.

CATION EXCHANGE

CASE STUDIES

Improving Aggregate Removal from a Monoclonal Antibody Feedstream Using High-Resolution CEX Chromatography

Background

Ever increasing performance demands in protein purification require more selective chromatography methods to effectively remove aggregates and other impurities. At high titer levels, aggregates often present a unique purification challenge. One emerging approach to address this challenge is the use of new, smaller-particle chromatography resins that are optimized for high resolution and capacity. Such resins can be particularly productive in challenging situations and during final polishing steps (He et al. 2010). Nuvia™ HR-S is a high-resolution resin. It features a hydrophilic polymer matrix with an open-pore structure designed for fast and efficient mass transfer and superior flow properties at high flow rates. In this case study, the performance of Nuvia HR-S was compared to that of a high-resolution agarose-based resin.

Method

Initial Separation of Aggregate from Monomer Using Nuvia HR-S Resin

Column: 2.1 ml, 0.7 x 5.6 cm

Step	Buffer	CV	Flow Rate, cm/hr
Equilibration	40 mM sodium acetate, pH 5 (buffer A)	3	310
Sample Loading	Treated mAb at pH 5	9.5	310
Elution 1	Gradient of buffer A to buffer A + 1.0 M NaCl	20	310
Elution 2	Buffer A + 1.0 M NaCl	2	310
Sanitization	1 N NaOH	5	310

This method applies to Figure 1.

Comparison of Nuvia HR-S Resin with a Commercial Small Particle Size CEX Resin

Column: 2.1 ml, 0.7 x 5.6 cm

Step	Buffer	CV	Flow Rate, cm/hr
Equilibration	40 mM sodium acetate, pH 5 (buffer A)	3	150
Sample Loading	Treated mAb at pH 5	9.5	150
Elution 1	Gradient of buffer A to buffer A + 0.4 M NaCl	20	150
Elution 2	Buffer A + 0.4 M NaCl	2	150
Sanitization	1 N NaOH	5	150

This method applies to Figure 3.

Results

Preparation of Aggregate and Monomer Test Solution

The in vitro generation of aggregates was designed to obtain a final composition of ~10% aggregates. Several initial attempts were made with varying ratios of monomeric to aggregated antibody. A final ratio of 2.5 volumes of monomeric antibody solution to 1 volume of aggregated antibody solution was chosen to yield a composition with the following properties:

A_{280} = 6.3 or 4.56 mg/ml
pH = 4.91
Conductivity = 2.6 mS/cm
Aggregate = 8.9%

A plot of log MW of six protein standards vs. their retention times yielded a straight line (R^2 = 0.96). Using this plot and the retention times of the aggregates, the apparent MW of the aggregate was calculated to be 386 kD.

Initial Separation of Aggregate from Monomer Using Nuvia HR-S

As shown in Figure 1, there was considerable overlap of the monomer and aggregate peaks (fractions (Fr) 17–22). Depending on the fraction pooling method, monomer recovery of 66–81% was achieved with an aggregate content of 0.46–0.85%. This observation is consistent with the premise that greater recovery results in higher aggregate content. The quality of the recovered pools (Figure 2) is shown in the overlay of the enlarged front shoulders of each SEC profile. The results demonstrated the feasibility of using Nuvia HR-S to separate aggregates from the monomer and showed that the extent of residual aggregates depended on the fractions included in the pool. Additionally, the overlap of monomer and aggregates in the Nuvia HR-S elution profile suggested that a lower flow rate and a shallower gradient (0.1–0.4 M NaCl) could be beneficial.

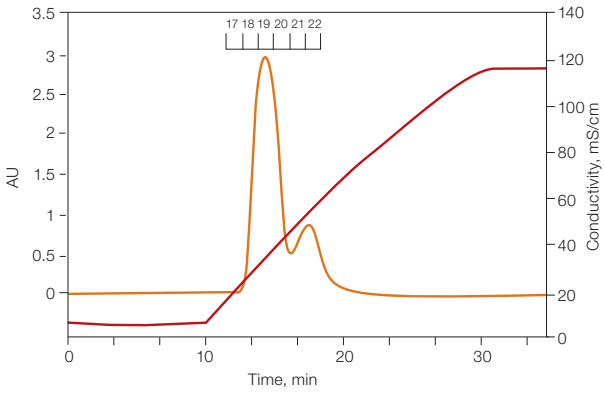


Fig. 1. Separation of aggregates from monomer by Nuvia HR-S before optimization. Absorbance (—); conductivity (—).

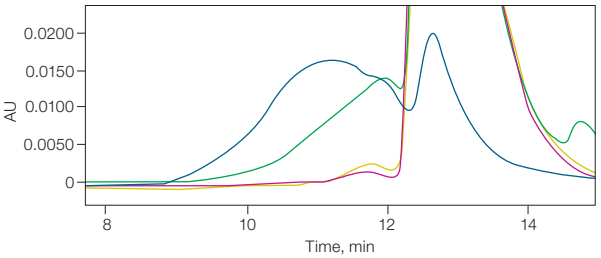


Fig. 2. SEC profiles. Load (—); selected pools (Fr 17+18+19+20) (—) and (Fr 17+18+19) (—); aggregated pool (Fr 21+22) (—).

Comparison of Nuvia HR-S with a Commercial Small Particle Size CEX Resin

A loading level of 46 mg IgG/ml resin was employed to challenge the binding capacity of Nuvia HR-S and Resin 1, and aggregate clearance by each resin was evaluated. A comparison of the elution profiles obtained from each chromatography run using the method conditions listed is shown in Figure 3. As indicated by the absorbance profile, the chromatography conditions resulted in a low yield for Resin 1 due to incomplete binding during the loading phase. In contrast, Nuvia HR-S was able to quantitatively retain the antibody before elution.

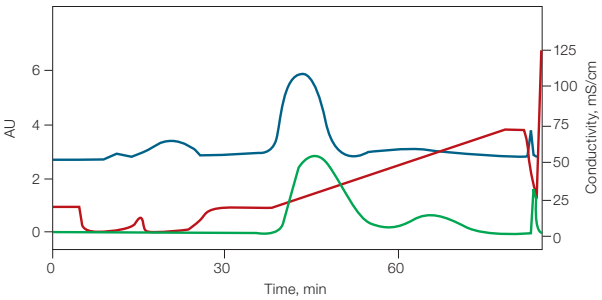


Fig. 3. Elution profiles after optimization. Nuvia HR-S (—); Resin 1 (—); conductivity (—).

Rather than subjectively determining the pertinent fractions, a strategy was designed to measure the aggregate content and the monomer recovery as fractions were progressively pooled during elution. As shown in Figure 4, both resins show an increase in aggregates as the pooling was extended to increase monomer recovery. While Nuvia HR-S was able to deliver a final aggregate content of <0.3% and a recovery of >80%, Resin 1 recovered less than 70% of total monomer at the same target aggregate content. This is due to the lower binding capacity of Resin 1, as confirmed by the increase in its absorbance during loading (Figure 3).

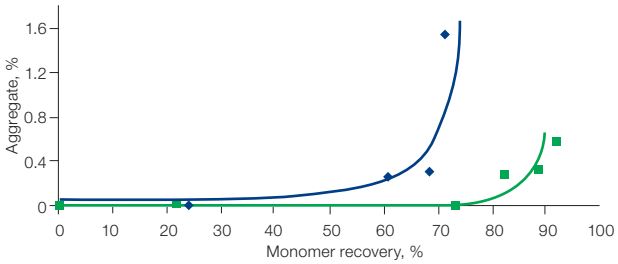


Fig. 4. Performance of Nuvia HR-S (■) vs. Resin 1 (◆).

Correlation between Monomer Recovery, Aggregate Content, and Target Conductivity

As depicted in Figure 4, aggregate recovery increases with increasing monomer recovery. In the Nuvia HR-S gradient elution, conductivity of the last fraction added to the pool was determined as the cutoff target of the pool. As shown, recovery is a function of the acceptable aggregate percentage and the target conductivity (Figure 5). Using the Fit Model function in JMP Software (SAS Institute Inc.), recovery could be correlated to those two measurements. A summary of actual and predicted data is shown in Figure 6.

The model yielded excellent correlation (R^2 = 0.94). This finding confirms the need to determine conductivity for elution so that both recovery and aggregate levels are within acceptable limits.

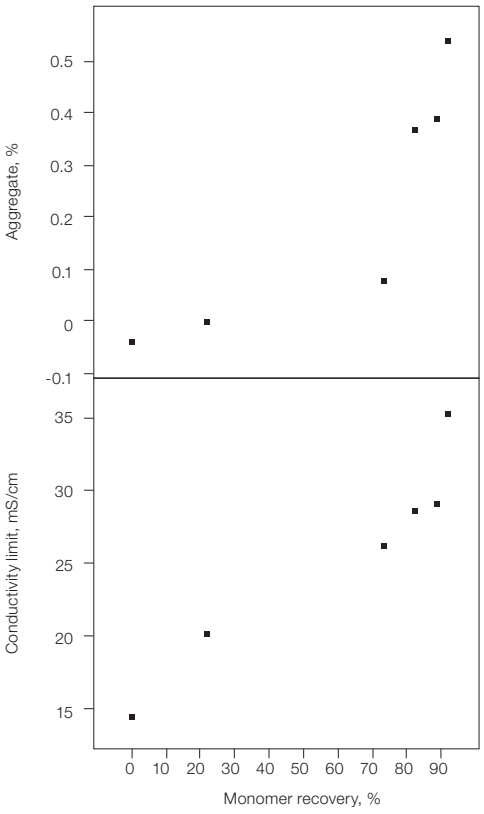
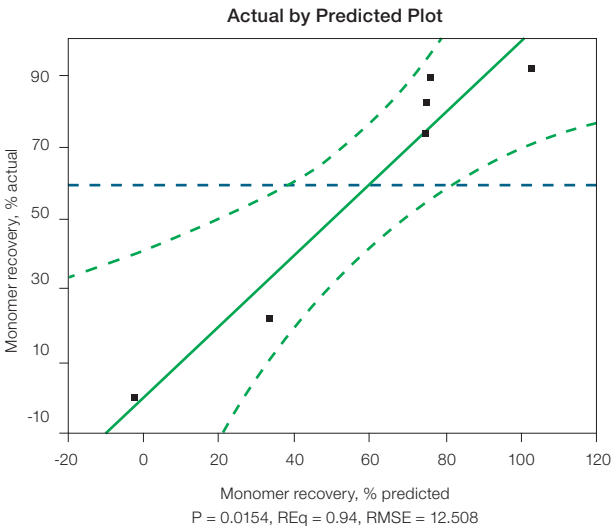


Fig. 5. Scatter plot matrix of aggregate content or conductivity vs. monomer recovery on Nuvia HR-S.



Summary of Fit

R^2	0.938098
R^2 adj	0.89683
Root mean square error	12.50828
Mean of response	59.78333
Observations (or sum wgts)	6

Fig. 6. Fit model of monomer recovery on Nuvia HR-S.

Summary

The data presented here demonstrate that Nuvia HR-S High-Resolution Cation Exchange Resin reduced aggregate content to <0.3% with >80% monomer recovery from a mAb feedstream containing 8.9% aggregates. In addition, Nuvia HR-S provides significantly higher recovery than a comparable agarose-based small-particle CEX resin. Finally, aggregate removal and final recovery are shown to be functions of buffer conductivity measured at the end of eluate collection.

References

He X et al. (2010). Nuvia S Media. BioProcess International 8, 59–61.

MIXED-MODE

CASE STUDIES

Mammalian Virus Purification

Background

Viruses can infect mammalian cells and cause diseases such as influenza, hepatitis, yellow fever, smallpox, and AIDS. Since some biotherapeutic products are produced using mammalian cell lines or plasma, the risk of viral contamination in these products is a concern and guidelines have been enforced to alleviate this risk. Chromatographic separation of viral particles from process intermediates is a key part of ensuring viral safety in biotherapeutics (ICH Expert Working Group 1999, Möritz 2005). Additionally, purification of viral particles is used extensively in the study and characterization of these infectious agents. Understanding aspects of a virus, such as how it infects host cells, uses the host cells for reproduction, and evades the host immune system, aids scientists in determining how to use viruses for research and therapy. In order to study a virus, a pure, high-quality infectious population is required. Conventional techniques for mammalian virus purification, for uses such as vaccine production or biological studies, can produce material of variable quality and quantity, often with significant loss of particle infectivity.

In this case study, ceramic hydroxyapatite media is used for purification of a wide variety of mammalian viruses (Table 1). Viral activity was determined using the assays shown in Table 2. Chromatography using ceramic hydroxyapatite media is simple, easily scalable, and results in a concentrated preparation of highly active virus.

Table 1. Viral type and size.

Virus	Family	Genus	Genome	Envelope	Size, nm
Dengue	Flaviviridae	Flavivirus	ssRNA	+	50
Japanese encephalitis	Flaviviridae	Flavivirus	ssRNA	+	50
Influenza	Orthomyxoviridae	Influenzavirus	ssRNA	+	80–120
Mouse hepatitis	Coronaviridae	Coronavirus	ssRNA	+	100–150
Adenovirus	Adenoviridae	Mastadenovirus	dsDNA	–	90
Poliovirus	Picornaviridae	Enterovirus	ssRNA	–	30
Feline calicivirus	Caliciviridae	Vesivirus	ssRNA	–	30–38

Table 2. Detection methods used for viral activity.

Detection Method	Virus
Hemagglutination (HA) test	Dengue, influenza, adenovirus
Plaque assay	Japanese encephalitis
50% tissue culture infective dose ($TCID_{50}$)	Poliovirus, feline calicivirus, mouse hepatitis

Method

Purification on CHT™ Ceramic Hydroxyapatite Media, Type II, 40 µm

Column: 0.6 ml, 0.46 x 3.5 cm

Step	Buffer	CV	Flow Rate, cm/hr
Wash	600 mM sodium phosphate, pH 7.2 (buffer B)	8	350
Equilibration	10 mM sodium phosphate, pH 7.2 (buffer A)	16	350
Sample Loading	Virus preparation in buffer A	16	350
Wash	Buffer A	16	350
Elution 1	Gradient of buffer A to buffer B	24	350
Elution 2	Buffer B	8	350

This method applies to Figures 1A, 2, 3, 4, 6B, and 7A.

Purification on CHT Media, Type II, 40 µm

Column: 0.6 ml, 0.46 x 3.5 cm

Step	Buffer	CV	Flow Rate, cm/hr
Wash	600 mM sodium phosphate, pH 7.2 (buffer B)	8	35
Equilibration	10 mM sodium phosphate, pH 7.2 (buffer A)	16	35
Sample Loading	Virus preparation in buffer A	16	35
Wash	Buffer A	16	35
Elution 1	Gradient of buffer A to buffer B	24	35
Elution 2	Buffer B	8	35

This method applies to Figure 1B.

Purification on CHT Media, Type II, 40 µm

Column: 0.6 ml, 0.68 x 2.0 cm

Step	Buffer	CV	Flow Rate, cm/hr
Wash	400 mM sodium phosphate, pH 7.2 (buffer B)	8	160
Equilibration	10 mM sodium phosphate, pH 7.2 (buffer A)	16	160
Sample Loading	Virus preparation in buffer A	16	160
Wash	Buffer A	16	160
Elution 1	Gradient of buffer A to buffer B	24	160
Elution 2	Buffer B	8	160

This method applies to Figure 5.

Purification on CHT Media Type I, 40 µm

Column: 0.6 ml, 0.46 x 3.5 cm

Step	Buffer	CV	Flow Rate, cm/hr
Wash	600 mM sodium phosphate, pH 7.2 (buffer B)	8	350
Equilibration	10 mM sodium phosphate, pH 7.2 (buffer A)	16	350
Sample Loading	Virus preparation in buffer A	16	350
Wash	Buffer A	16	350
Elution 1	Gradient of buffer A to buffer B	24	350
Elution 2	Buffer B	8	350

This method applies to Figure 6A.

Purification on CFT™ Ceramic Fluoroapatite Media, Type II, 40 µm

Column: 0.6 ml, 0.46 x 3.5 cm

Step	Buffer	CV	Flow Rate, cm/hr
Wash	600 mM sodium phosphate, pH 7.2 (buffer B)	8	350
Equilibration	10 mM sodium phosphate, pH 7.2 (buffer A)	16	350
Sample Loading	Virus preparation in buffer A	16	350
Wash	Buffer A	16	350
Elution 1	Gradient of buffer A to buffer B	24	350
Elution 2	Buffer B	8	350

This method applies to Figures 6C and 7B.

Purification on MPC™ Ceramic Hydroxyfluoroapatite Media, 40 µm

Column: 0.6 ml, 0.46 x 3.5 cm

Step	Buffer	CV	Flow Rate, cm/hr
Wash	600 mM sodium phosphate, pH 7.2 (buffer B)	8	350
Equilibration	10 mM sodium phosphate, pH 7.2 (buffer A)	16	350
Sample Loading	Virus preparation in buffer A	16	350
Wash	Buffer A	16	350
Elution 1	Gradient of buffer A to buffer B	24	350
Elution 2	Buffer B	8	350

This method applies to Figure 6D.

Results

Dengue Virus

Figure 1A shows the recovery of dengue virus type 2 from cell culture fluid. HA activity was recovered near the end of the gradient, separated from the bulk of A_{280}-absorbing material and from dsDNA (Kurosawa et al. 2012b). Figure 1B demonstrates that decreasing the flow rate by tenfold improves the sharpness of the elution peaks and, hence, separation. In both cases, recovery of HA activity was greater than 95%. Recent studies have indicated that adsorption of dengue virus particles to the surface of CHT Type II Media is similar to their adsorption to cells (Saito et al. 2013).

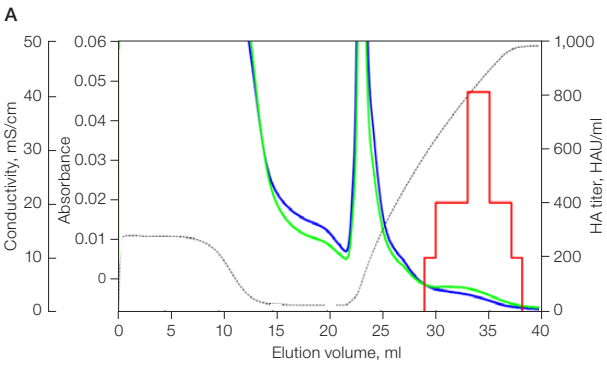


Fig. 1. Chromatograms of the separation of dengue virus type 2 by CHT Type II Media. A, flow rate at 1.0 ml/min; **B**, flow rate at 0.1 ml/min. UV absorbance at 260 nm (—), UV absorbance at 280 nm (—); conductivity (—); viral activity in HA test (—).

Other serotypes of dengue virus also bound to and eluted from CHT Type II Media. The approximate elution points in the sodium phosphate gradient for each serotype are shown in Table 3. Types 2 and 4 eluted at roughly the same position.

Table 3. Elution points of dengue serotypes in a sodium phosphate gradient.

Virus Serotype	Approximate Elution Point, mM
1	250
2	450
4	425

Influenza Virus

Chromatography of influenza virus A/Beijing/262/95 and A/Panama/2007/99 (Schickli et al. 2001) cultured in the presence of 0.02% and 0.20% BSA, respectively, is shown in Figure 2.

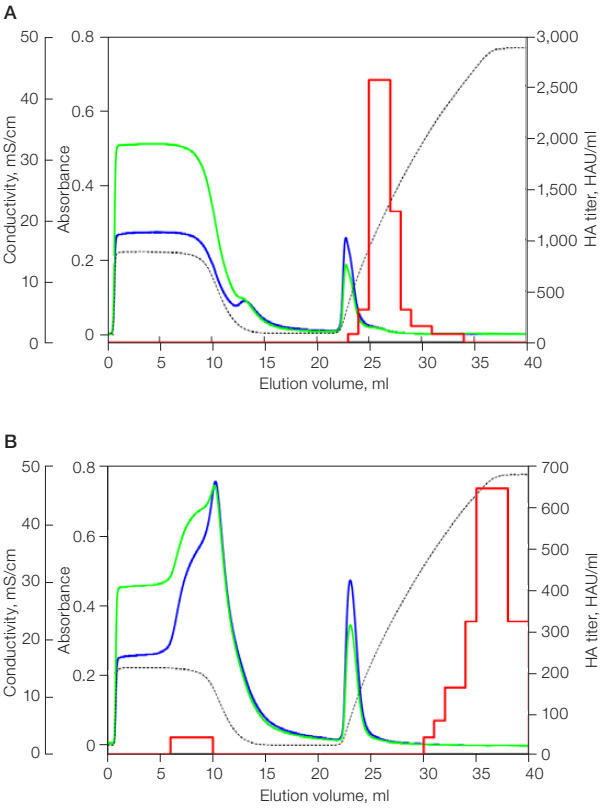


Fig. 2. Chromatography of influenza virus. A, A/Beijing/262/95; **B**, A/Panama/2007/99. UV absorbance at 260 nm (—); UV absorbance at 280 nm (—); conductivity (**--**); viral activity in HA test (—).

HA activity is separated from a small BSA peak and a significant amount of material that did not bind to the column. Recovery, as measured by the HA assay, was 98% for the A/Beijing/262/95 virus. Higher concentrations of sodium phosphate are required to elute the A/Panama/2007/99 virus. In addition, the retention time was not affected by the source (allantoic fluid vs. cell culture; data not shown).

Mouse Hepatitis Virus

Mouse hepatitis virus (MHV) is a coronavirus (CoV), a genus that includes SARS-CoV. Two strains of MHV (MHV-NuU and MHV-S) (Hirano et al. 1981) were applied and bound to CHT Type II Media. Both were eluted at 26–28 minutes in the gradient (Figure 3).

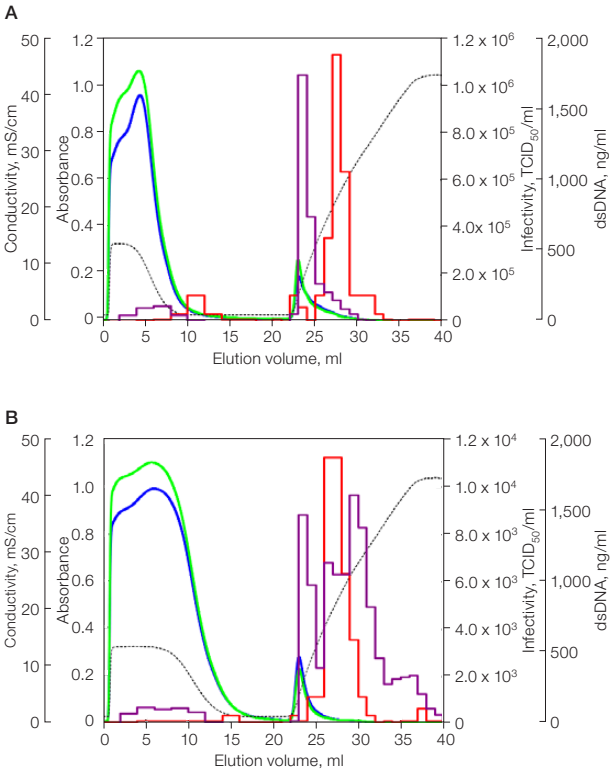


Fig. 3. Chromatography of two strains of MHV (A, MHV-NuU; B, MHV-S). UV absorbance at 260 nm (—); UV absorbance at 280 nm (—); conductivity (**--**); viral infectivity in $TCID_{50}$ (—); dsDNA (—). Culture fluid contained 10% fetal bovine serum (FBS).

Nonenveloped Viral Particles

Nonenveloped viral particles can be purified by ceramic hydroxyapatite chromatography in the same way as enveloped viruses. Adenovirus (AdV) type 27, feline calicivirus (FCV) A391 (Hirano et al. 1986), and poliovirus (PV) Sabin type 2 all adsorbed to CHT Type II Media (Figure 4), although they showed different elution times.

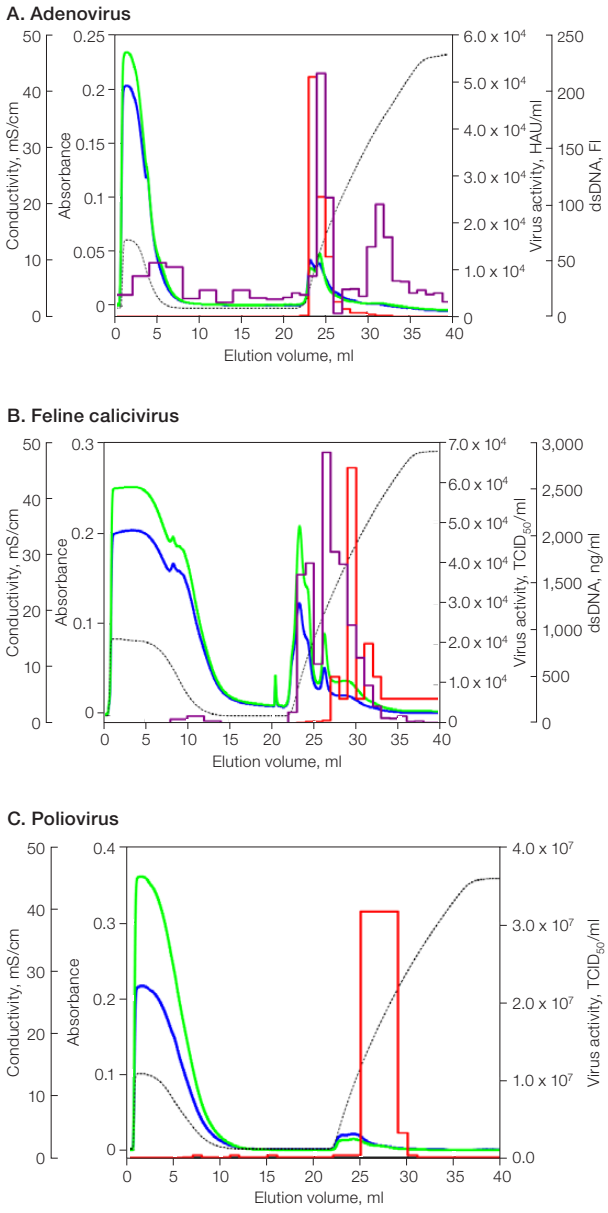


Fig. 4. Chromatograms of the separation of cell lysate (A) or culture fluid (B, C) containing nonenveloped viral particles by CHT Ceramic Hydroxyapatite Type II Media. A, AdV type 27; **B**, FCV A391; **C**, PV Sabin type 2. UV absorbance at 260 nm (—); UV absorbance at 280 nm (—); conductivity (**--**); viral activity (AdV in HA test, FCV and PV in $TCID_{50}$) (—); dsDNA (—). Cell culture fluid contained 10% FBS. FI, fluorescence intensity.

Japanese Encephalitis Virus

Japanese encephalitis virus (JEV) chromatography is shown in Figure 5 (Kurosawa et al. 2009, 2012a). Irrespective of the source or strain, the virus elutes at approximately 350 mM sodium phosphate (note that the gradient in these two cases is 10–400 mM and the column size is 6.8 x 20 mm). Again, there is good separation between protein contamination and the virus.

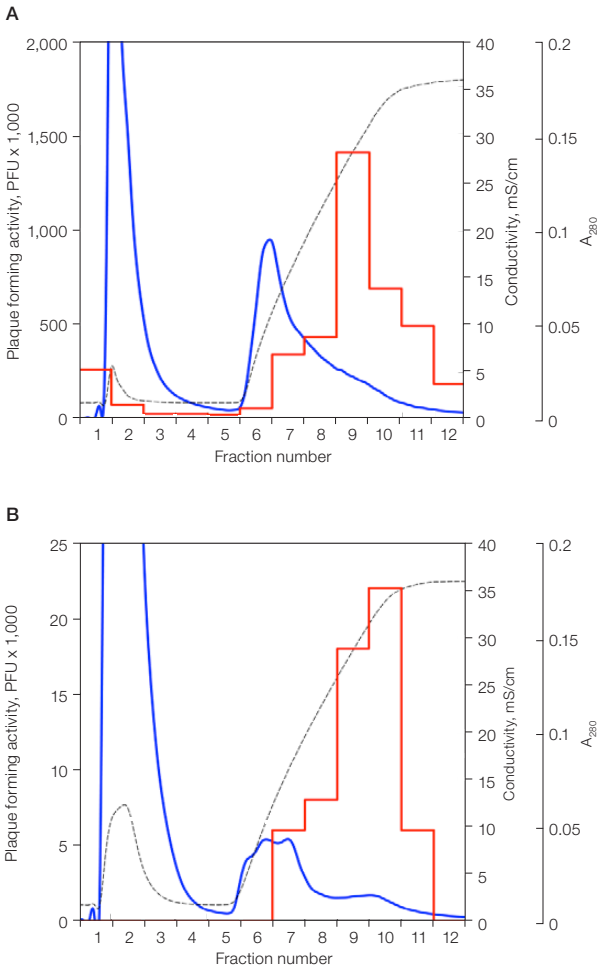


Fig. 5. JEV chromatography at pH 7.0. A, mouse brain homogenate infected with JEV JaGAr01; **B**, cell culture fluid of JEV Beijing. UV absorbance at 280 nm (—); conductivity (**--**); infectious activity in plaque assay (—).

Note: Figure 5A is modified from Kurosawa et al. 2012a.

Effect of Hydroxyapatite Type on Separation

Figure 6 shows the separation of dengue virus type 2 from cell culture contaminants on four apatites: CHT Type I, CHT Type II, CFT Type II, and MPC Media. Yields were 80% or higher for each media type except for MPC, where the yield was 50%. Although binding and elution was achieved on all four media, the separation of virus from impurities was best on CHT Type II Media. Figure 7 shows a similar study using CHT Type II and CFT Type II Media for the purification of poliovirus, with recoveries of 88% and 102%, respectively. These results illustrate the importance of choosing the appropriate media for the separation in question.

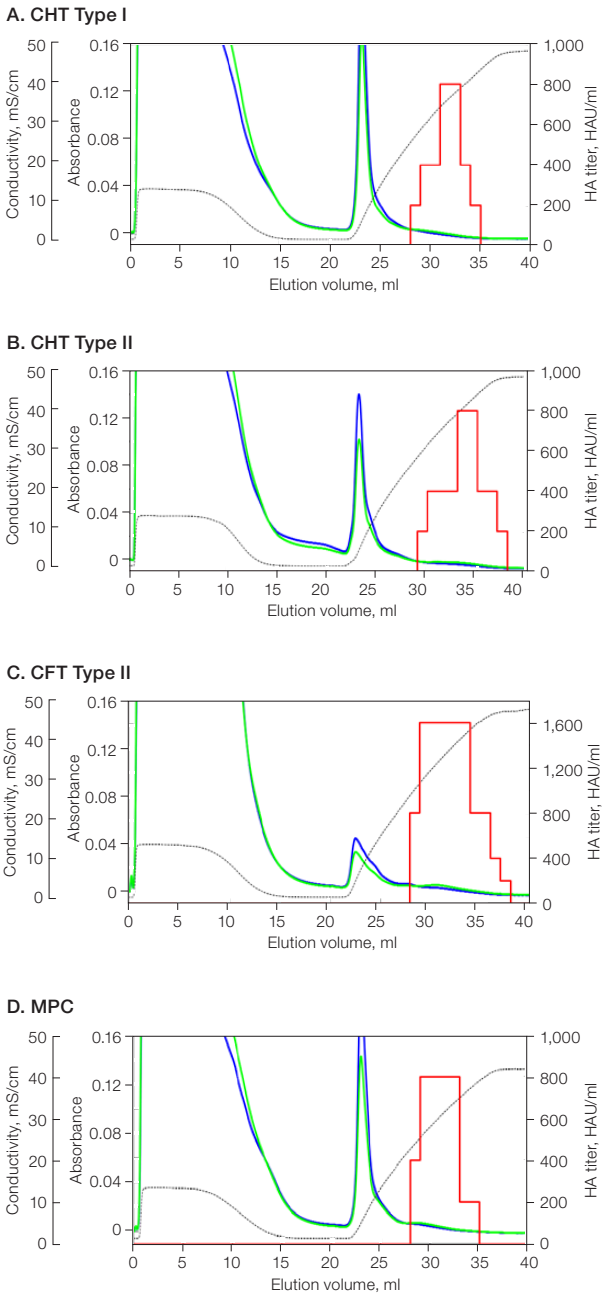


Fig. 6. Chromatograms of dengue virus type 2. A, CHT Type I Media; **B**, CHT Type II Media; **C**, CFT Type II Media; **D**, MPC Media. UV absorbance at 260 nm (—); UV absorbance at 280 nm (—); conductivity (**--**); viral activity in HA test (—).

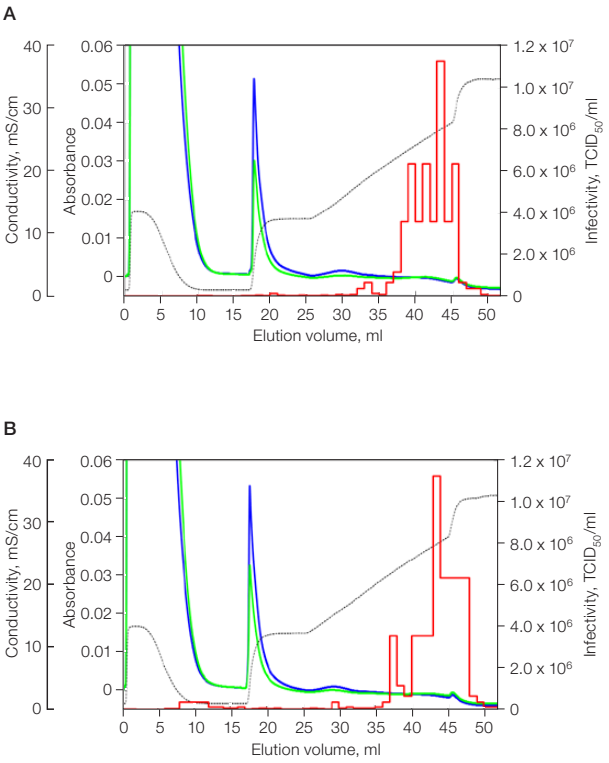


Fig. 7. Chromatograms of culture fluid of polio virus at pH 6.4. A, CHT Type II Media; **B**, CFT Type II Media. UV absorbance at 260 nm (—); UV absorbance at 280 nm (—); conductivity of elution buffer (**--**); infectious activity in $TCID_{50}$ (—). Note: the gradient in these two cases is 150–450 mM at pH 6.4 for 20 ml.

Summary

Using ceramic hydroxyapatite media provided high purity, recovery, and viral activity for seven mammalian viruses of varying size and belonging to different families. We have shown that, in at least one case, slowing the flow rate and decreasing the gradient slope allowed better purification of viral particles on CHT Type II Media, signifying the importance of determining the best settings for such factors when using apatite media. Testing different apatites is significant for determining which media type will work best for a specific virus. A larger pore size, as provided by the CHT Type II Media, allowed better separation of the dengue virus from contaminants compared to other apatite media.

Of equal significance, the use of ceramic hydroxyapatite media is simple and provides reproducible results, allowing an alternative to the conventional methods of viral purification.

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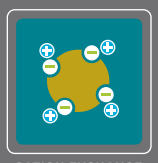
CATION EXCHANGE


ANION EXCHANGE

CASE STUDIES

Rapid Single-Step Purification of Proteins from Whey

Background

Whey proteins play a vital role in the formulation of food supplements because of numerous desirable nutritional and functional properties. This case study depicts the establishment of a process workflow for whey purification using Nuvia™ S and Nuvia Q Ion Exchange Resins. The major whey proteins are α-lactalbumin (ALA), β-lactoglobulin (BLG), bovine serum albumin (BSA), and bovine immunoglobulins (Hahn et al. 1998) (Table 1). Minor proteins such as lactoperoxidase, lactoferrin, and proteose-peptone account for the other protein components in whey.

Table 1. Protein composition of bovine whey.

Protein	Average Concentration, g/L	Proportion in Whey, %	Molecular Weight	Isoelectric Point
ALA	1.5	22	14,200	4.7–5.1
BLG	3–4	51	18,400	5.2
BSA	0.3–0.6	6.6	65,000	4.9
IgG, IgA, IgM	0.6–0.9	11	150,000–900,000	5.8–7.3
Lactoperoxidase	0.06	<1	78,000	9.6
Lactoferrin	0.05	<1	78,000	8.0
Proteose-peptone	0.5	7	4,000–20,000	—

The objective of this study was to develop a separation process to fractionate usable proteins from whey (Figure 1). First, total whey protein isolate (WPI) was purified because of its high-end functional advantages, as evidenced by a demand from nutrition and health segments. Second, ALA and BLG were purified because of their value in infant formula and confections, respectively.

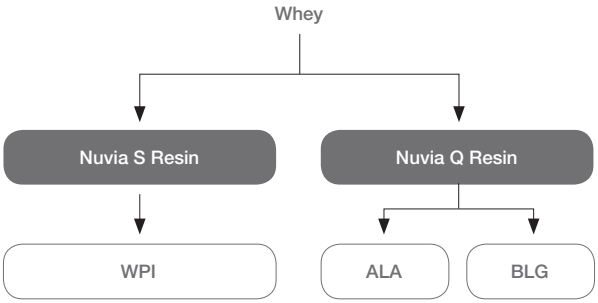

Fig. 1. Workflow for protein purification from whey with Nuvia S and Nuvia Q Resins.

CEX Chromatography Using Nuvia S Resin

Column: 1 ml, 0.7 x 2.7 cm

Step	Buffer	CV	Flow Rate, cm/hr
Equilibration	0.04 M sodium lactate, pH 4.0 (buffer A)	3	150
Sample Loading	Whey concentrate diluted 1:10 in buffer A, 1.2 mM filtered	20	150
Wash 1	Buffer A	10	150
Elution	0.04 M sodium phosphate, pH 8.0	20	150
Wash 2	1 M NaCl	3	150
Wash 3	1 N NaOH	3	150
Equilibration	0.2 M sodium lactate, pH 4.0	3	150
Re-Equilibration	Buffer A	5	150

AEX Chromatography Using Nuvia Q Resin

Column: 1 ml, 0.7 x 2.7 cm

Step	Buffer	CV	Flow Rate, cm/hr
Equilibration	0.02 M sodium phosphate, pH 6.0 (buffer A)	3	150
Sample Loading	Whey concentrate diluted 1:10 in buffer A, 1.2 mM filtered	5	150
Wash	Buffer A	4	150
Elution 1	Gradient of buffer A to buffer A + 0.1 M NaCl	10	150
Elution 2	Buffer A + 0.1 M NaCl	5	150
Elution 3	Gradient of buffer A + 0.1 M NaCl to buffer A + 0.3 M NaCl	10	150
Elution 4	Buffer A + 0.3 M NaCl	5	150
Elution 5	Gradient of buffer A + 0.3 M NaCl to buffer A + 0.9 M NaCl	10	150
Elution 6	Buffer A + 0.9 M NaCl	10	150

Results

Preparation of Whey Protein Isolate

As defined by the Whey Protein Institute (wheyoflife.org), WPI is the most pure and concentrated form of whey protein with few or no lipids or lactose. Since the majority of the proteins have isoelectric points above 4.7, loading of whey at pH 4.0 onto Nuvia S Resin resulted in the capture of these proteins (Etzel 2004, Hahn et al. 1998). Lipids, lactose, and unbound proteins remain in the flowthrough and the wash. As shown in Figure 2, a single peak was collected upon elution with pH 8.0 elution buffer. Capacity of the Nuvia S Column, based on the measurement of the protein content in the eluate pool, was 60 ± 2 g/L (60 CV/hr, n = 4). As shown in Figure 3, proteins in WPI and in whey are similar. Interestingly, densitometry of the major species, including ALA, BLG, and BSA, in whey and WPI showed nearly identical distributions.

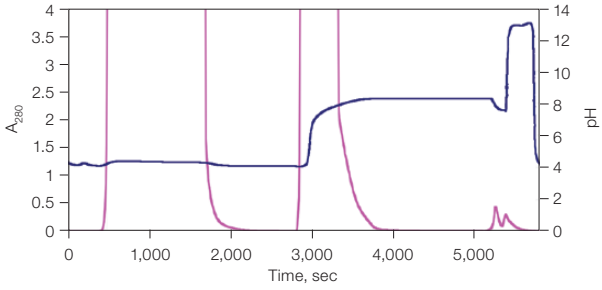

Fig. 2. CEX chromatography of 80 mg of crude whey on a Nuvia S Column. Column: 7 x 27 mm; 2 ml fractions were collected; flow rate: 60 CV/hr; buffer A: 0.04 M Na lactate, pH 4.0; buffer B: 0.04 M Na phosphate, pH 8.0. A_{280} (—); pH (—).

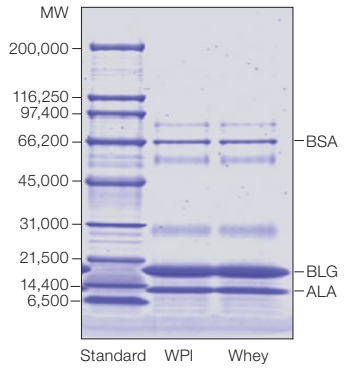

Fig. 3. SDS-PAGE analysis of WPI and whey. Whey protein and WPI (10 mg) along with Bio-Rad SDS-PAGE standards were run on a 4–20% Criterion™ TGX™ Precast Gel. Proteins were stained using BioSafe™ Coomassie Stain.

Anion Exchange Resin Selection and Optimization

ALA and BLG account for about 75% of the proteins in whey. These two model proteins were therefore selected for further experimentation. In an initial screen, Nuvia Q Resin was compared to two resins with high binding capacity (resin A and resin B). The resins were tested using a small mixture of ALA and BLG (10% CV) that was eluted using a shallow NaCl gradient (0–0.3 M). This permits rapid and uniform evaluation of process parameters, but further optimization will be needed for the selected resin. The resulting chromatograms from this screening experiment were compared (Figure 4). All three resins produced two equally well-resolved peaks. Peaks of resin A eluted sooner, suggesting weaker AEX properties than Nuvia Q Resin and resin B. The bead size of resin A is the smallest of the three resins, which may lead to higher column pressure upon scale-up. Additionally, rigidity, cleanability, capacity, and cost will have to be factored in when choosing the appropriate resin for large-scale purification.

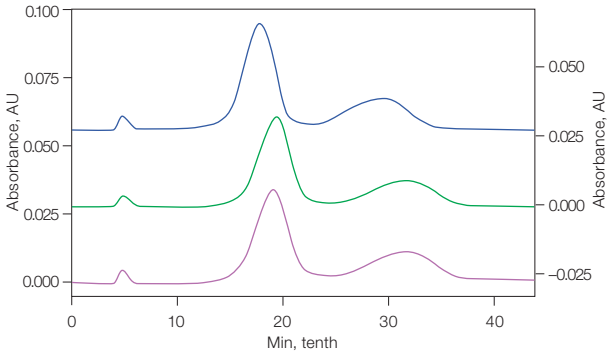

Fig. 4. ALA and BLG purification chromatograms on three anion exchangers. Comparison of Nuvia Q Resin (—), resin A (—), and resin B (—) performance.

Purification of ALA and BLG from Whey

To minimize processing costs, a purification scheme for ALA and BLG from whey ideally should require a single chromatographic step to purify both proteins. Initial studies (Kim et al. 2003, Gerberding and Byers 1998) demonstrated the removal of the major whey proteins (ALA, BSA, and BLG) in a segmented gradient (data not shown). Further work was performed to optimize these conditions. The purification profile of each protein was first identified by injecting the purified protein alone and then a mixture containing the three purified proteins. As expected, separation of the mixture paralleled that of the individual proteins. When whey was used as the feed stock, two major pools were resolved upon adsorption and elution with the segmented gradient (Figure 5).

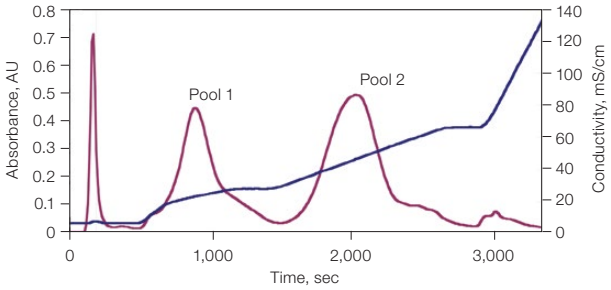

Fig. 5. AEX chromatography of 8 mg of crude whey on a 7 x 27 mm Nuvia Q Column. Fractions of 2 ml were collected; flow rate: 60 CV/hr; buffer: 0.02 M Na phosphate, pH 6.0; segmented gradient: 0–0.1 M NaCl, 0.1–0.3 M NaCl, and 0.3–0.9 M NaCl. A_{280} (—); conductivity (—).

SDS-PAGE analysis (Figure 6) of the fractions showed two peaks, one containing predominantly ALA (Pool 1) and the other containing predominantly BLG (Pool 2). The corresponding ALA and BLG standards are also shown in the gel scan. Purities of the two whey-derived proteins in each peak were estimated by densitometry to be at least 85%.

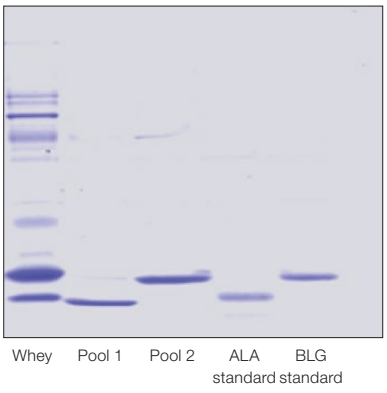

Fig. 6. SDS-PAGE analysis of whey, purified ALA (Pool 1), and purified BLG (Pool 2) against protein standards.

Summary

Nuvia S and Nuvia Q Resins, two novel high-capacity resins for IEX chromatography, allow efficient enrichment of proteins from crude mixtures. The feasibility to prepare WPI, purified ALA, and purified BLG has been demonstrated. Because of the high-throughput properties of Nuvia Resins, a rapid single-step purification of proteins from whey is possible.

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MIXED-MODE

CASE STUDIES

Mixed-Mode Chromatography for mAb S Aggregate Removal

Background

mAbs remain a predominant class of therapeutic protein products on the market because of their wide range of applications in disease treatment and diagnosis. Over the years, upstream technology advancements have helped improve the titer of target antibodies, from merely 1 g/L two decades ago to 10–13 g/L in fed-batch processes and up to 25 g/L in perfusion cultures today (Gronemeyer et al. 2014). However, these advancements have often adversely affected impurity composition and concentration upon harvest. This has had a significant impact on downstream processing. Elevated levels of antibody aggregates are often associated with the overproduction of mAbs due to the mispairing of disulfide bonds and the unfolding or denaturation of drug molecules at cell growth temperatures (≥25°C) (Rathore et al. 2013).

The strong acidic elution conditions needed for Protein A affinity chromatography, commonly used for the capture of mAbs from clarified tissue culture fluid, can trigger structural changes and promote the oligomerization of pH-sensitive molecules. Additional column chromatography steps are necessary for the clearance of such product-related impurities. The separation of mAb monomers and aggregates has been a major challenge in downstream manufacturing as these molecules exhibit very similar physical and chemical properties and sometimes identical chromatographic behavior. Baseline separation of these mAb species is often difficult to achieve, and extensive process development is required to balance the purity and yield of the monomeric mAbs.

The molecule of interest in this present study, mAb S, was harvested from Chinese hamster ovary (CHO) cell culture. Typically, a Protein A affinity chromatography eluate contains a mixture of mAb S monomers (~75%) and aggregates (dimers and other oligomers, ~25%). We separated the mAb S monomers from the aggregates using three mixed-mode chromatography media, CHT™ Ceramic Hydroxyapatite, Capto adhere, and Capto adhere ImpRes, which have all been extensively researched for their applications in mAb aggregate clearance (Gagnon 2009). We compared the purity and recovery of monomeric mAb S purified with these chromatography media. Our results underscore the importance of screening multiple media to develop an efficient downstream production process with the highest possible target purity and recovery.

Method

Affinity Chromatography Using UNOsphere SUPrA™ Resin

Column: 1 ml, 0.56 x 4 cm

Step	Buffer	CV	Flow Rate, cm/hr
Equilibration	1x PBS, pH 7.3	10	300
Sample Loading	CHO cell culture harvest	~20 mg in 20 ml	300
Wash	1x PBS, pH 7.3	10	300
Elution	100 mM glycine, pH 3.5	10	300

Mixed-Mode Chromatography Using CHT Ceramic Hydroxyapatite Media, Type I, 40 µm

Column: 1 ml, 0.56 x 4 cm

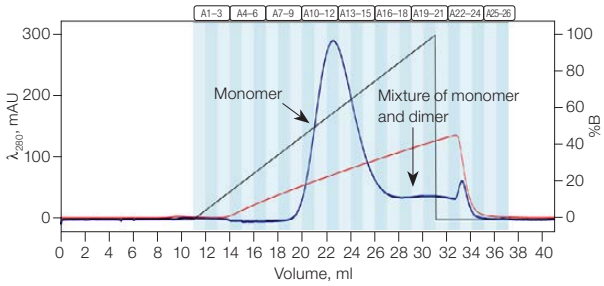
Step	Buffer	CV	Flow Rate, cm/hr
Equilibration	10 mM sodium phosphate, pH 7.0	10	300
Sample Loading	UNOsphere SUPrA eluate (mAb) adjusted to pH 7.0	2.8 mg in 1 ml	300
Wash	10 mM sodium phosphate, pH 7.0	5	300
Elution	10 mM sodium phosphate, 550 mM NaCl, pH 7.0	15	300

This method applies to Figure 2.

Results

The mAb S aggregates were well separated from the monomer using the CHT Column with a 0–1,000 mM sodium chloride gradient. The bulk of the mAb S monomer was eluted at ~50%B, which is equivalent to ~500 mM sodium chloride (Figure 1A). Elution of the monomer was complete at ~75%B or 750 mM sodium chloride, and increasing the salt concentration resulted in leaching of the undesired mAb S oligomers from the column (Figure 1B). Step elution of mAb S was then performed using a sodium phosphate buffer containing 550 mM sodium chloride (Figure 2). Fractions A5 to A9 were pooled, and the monomer content in this eluate was determined to be 99.5% with an overall mAb S monomer recovery of 82.7%.

A



Column:	1 ml CHT packed in a Bio-Scale™ Mini Cartridge (0.56 x 4 cm)	
Sample:	2.8 mg affinity-purified mAb S in 1 ml 10 mM sodium phosphate, pH 7.0	
Flow rate:	300 cm/hr	
Equilibration:	10 mM sodium phosphate, pH 7.0, 10 CV	
Post-loading wash:	10 mM sodium phosphate, pH 7.0, 5 CV	
Elution:	Buffer A	10 mM sodium phosphate, pH 7.0
	Buffer B	10 mM sodium phosphate, 1 M sodium chloride, pH 7.0
	A to B linear gradient, 0–100%B, 20 CV	

B

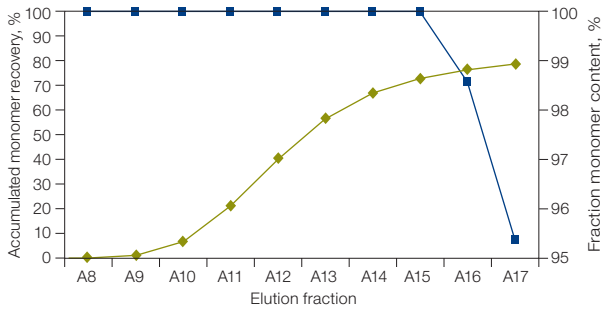


Fig. 1. Purification of mAb S on a CHT Column. A, removal of mAb S aggregates using a 0–1,000 mM sodium chloride gradient. OD 280 (—); conductivity (—); %B (—). The blue vertical bands represent where fractions were collected. **B**, mAb S monomer content in individual elution fractions (—■—) and accumulated monomer recovery in pooled elution fractions (—◆—).

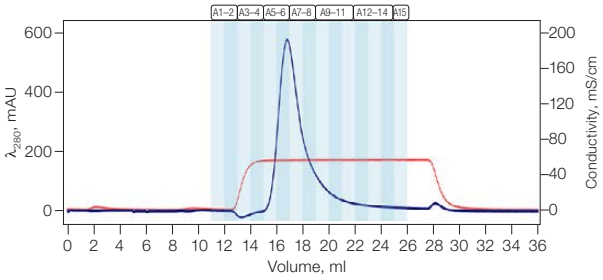
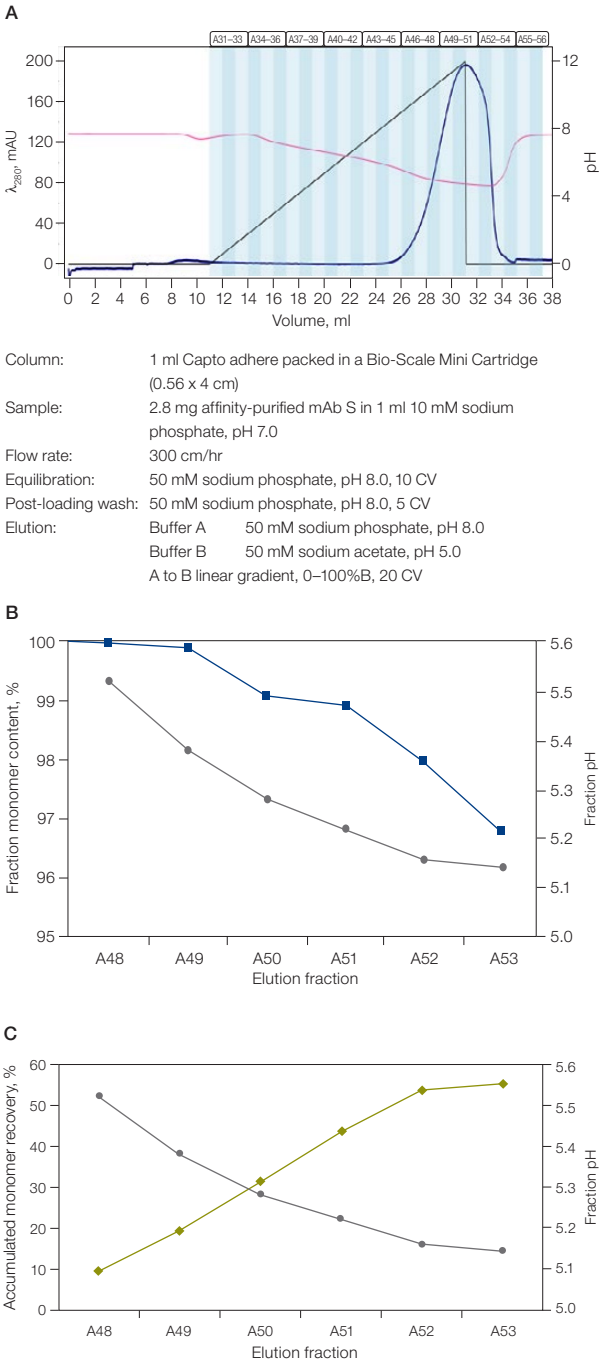


Fig. 2. Elution of mAb S monomers from a CHT Column. Elution was carried out using 10 mM sodium phosphate and 550 mM sodium chloride, pH 7.0. Fractions A5 to A9 were pooled. OD 280 (—); conductivity (—). The blue vertical bands represent where fractions were collected.

Preliminary DoE studies were performed to screen for mAb S purification conditions with Capto adhere Media. Binding was maximal with buffers containing ≤50 mM sodium chloride at pH above 8.0. mAb S was only partially eluted from the column under a modest acidic condition (pH 5.0). At pH 4.0, all mAb S species were eluted from the Capto adhere Column without differentiation of monomer and higher molecular weight species. The addition of sodium chloride to the elution buffer further hampered target protein recovery, which indicates a strong hydrophobic interaction between target molecules and this hydrophobic AEX media (data not shown). A gradient of pH 8–5 was therefore employed in an effort to elute mAb S monomers selectively from the Capto adhere Column (Figure 3A). Dependency of monomer content and elution pH was observed, with the later fractions containing significantly more high molecular weight species (Figure 3B) and exhibiting a plateau of mAb S monomer recovery (Figure 3C).



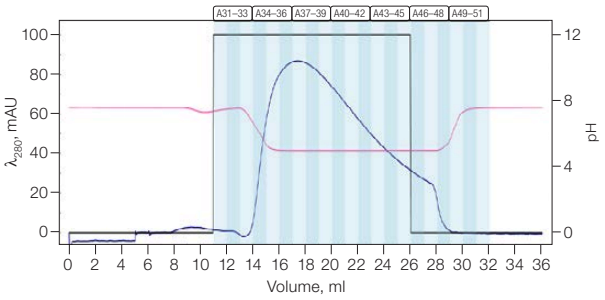
Column: 1 ml Capto adhere packed in a Bio-Scale Mini Cartridge (0.56 x 4 cm)
Sample: 2.8 mg affinity-purified mAb S in 1 ml 10 mM sodium phosphate, pH 7.0
Flow rate: 300 cm/hr
Equilibration: 50 mM sodium phosphate, pH 8.0, 10 CV
Post-loading wash: 50 mM sodium phosphate, pH 8.0, 5 CV
Elution: Buffer A 50 mM sodium phosphate, pH 8.0
Buffer B 50 mM sodium acetate, pH 5.0
A to B linear gradient, 0–100%B, 20 CV

Fig. 3. Purification of mAb S on a Capto adhere Column. A, removal of mAb S aggregates using a pH 8–5 gradient. OD 280 (—); pH (—); %B (—). The blue vertical bands represent where fractions were collected. **B**, mAb S monomer content in individual elution fractions (—■—) and its correlation with fraction pH (—●—). **C**, accumulated mAb S monomer recovery in pooled elution fractions (—◆—) and its correlation with fraction pH (—●—).

To further assess the effect of the elution pH on final mAb S monomer purity and recovery, step elutions under various pH conditions were performed. The results are summarized in Table 1. The chromatography of mAb S elution at pH 5.5 is shown in Figure 4. The purity of the product was found to be very sensitive to the pH of the elution buffer. A minor pH drop, from 5.5 to 5.4, resulted in a tripling of the aggregate content in the eluate. The pooled product from a pH 5.4 step elution failed the quality control (QC) specification of ≥99.5% monomer content. Furthermore, despite the fact that 14 CV of eluate were collected, monomer recovery was only ~53%.

Table 1. Pooled elution fractions from the Capto adhere Column using 50 mM sodium acetate buffer at different pH.

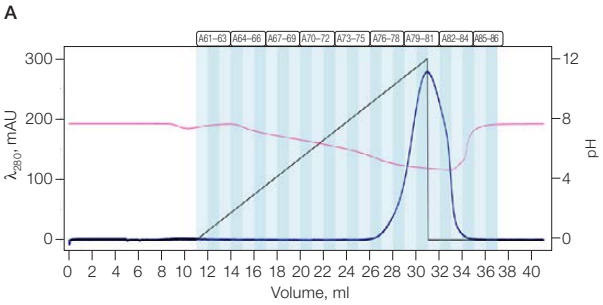
Elution pH	mAb S Monomer Content, %	mAb S Monomer Recovery, %	Eluate Volume, CV
5.2	96.9	76.9	14
5.3	98.1	64.9	14
5.4	98.8	52.5	14
5.5	99.7	48.8	14



Column: 1 ml Capto adhere packed in a Bio-Scale Mini Cartridge (0.56 x 4 cm)
Sample: 2.8 mg affinity-purified mAb S in 1 ml 50 mM sodium phosphate, pH 8.0
Flow rate: 300 cm/hr
Equilibration: 50 mM sodium phosphate, pH 8.0, 10 CV
Post-loading wash: 50 mM sodium phosphate, pH 8.0, 5 CV
Elution: 50 mM sodium acetate, pH 5.5, 15 CV

Fig. 4. Elution of mAb S monomers from a Capto adhere Column. Elution was carried out using 50 mM sodium acetate, pH 5.5. Fractions A35 to A48 were pooled. OD 280 (—); pH (—); %B (—). The blue vertical bands represent where fractions were collected.

Compared with Capto adhere, the binding of mAb S by Capto adhere ImpRes was more sensitive to the concentration of sodium chloride in the buffer. However, the elution of mAb S from this mixed-mode media seemed to be solely dependent on the pH of the buffer, as revealed by DoE screening (data not shown). Again, a pH gradient was used to resolve mAb S monomers from aggregates (Figure 5A). The pH of the elution fractions, monomer content, and monomer recovery were analyzed and the results are shown in Figures 5B and 5C.



Column:	1 ml Capto adhere ImpRes packed in a Bio-Scale Mini Cartridge (0.56 x 4 cm)
Sample:	2.8 mg affinity-purified mAb S in 1 ml 50 mM sodium phosphate, pH 8.0
Flow rate:	300 cm/hr
Equilibration:	50 mM sodium phosphate, pH 8.0, 10 CV
Post-loading wash:	50 mM sodium phosphate, pH 8.0, 5 CV
Elution:	Buffer A 50 mM sodium phosphate, pH 8.0 Buffer B 50 mM sodium acetate, pH 5.0 A to B linear gradient, 0–100%B, 20 CV

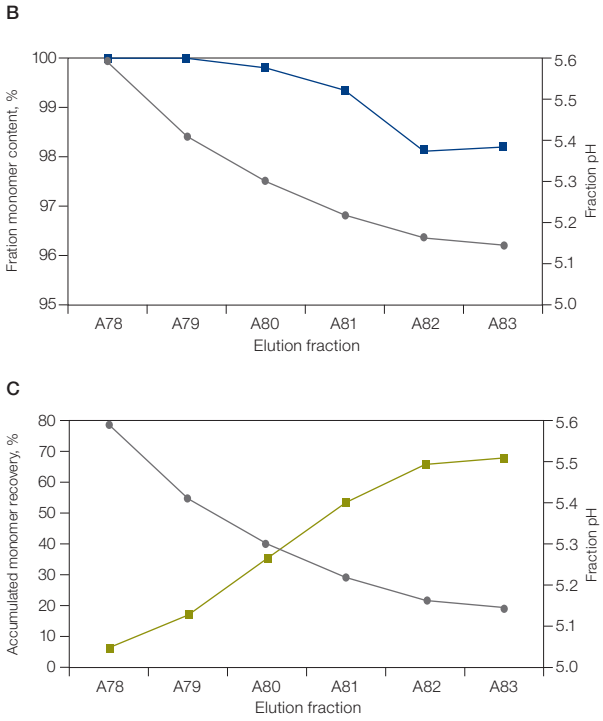
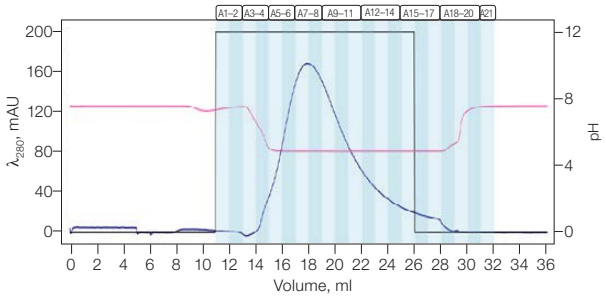


Fig. 5. Purification of mAb S on a Capto adhere ImpRes Column. **A**, removal of mAb S aggregates using a pH 8–5 gradient. OD 280 (—); pH (—); %B (—). The blue vertical bands represent where fractions were collected. **B**, mAb S monomer content in individual elution fractions (–■–) and its correlation with fraction pH (–●–). **C**, accumulated mAb S monomer recovery in pooled elution fractions (–■–) and its correlation with fraction pH (–●–).

Buffers at different pH were used to elute mAb S from the Capto adhere ImpRes Column in step mode (Table 2). Figure 6 depicts the chromatogram resulting from the elution of mAb S with 50 mM sodium acetate (pH 5.4). Overall, Capto adhere ImpRes offered better recovery of mAb S monomer than Capto adhere. The purity of mAb S monomer in the eluate met the QC specification of ≥99.5% monomer content and was consistent at pH 5.4 and higher; however, monomer recovery was only 50–62% after 14 CV of eluate were collected under these conditions.

Table 2. Pooled elution fractions from the Capto adhere ImpRes Column with 50 mM sodium acetate buffer at different pH.

Elution pH	mAb S Monomer Content, %	mAb S Monomer Recovery, %	Eluate Volume, CV
5.3	98.6	68.3	14
5.4	99.6	61.7	14
5.5	99.6	50.5	14



Column:	1 ml Capto adhere ImpRes packed in a Bio-Scale Mini Cartridge (0.56 x 4 cm)
Sample:	2.8 mg affinity-purified mAb S in 1 ml 50 mM sodium phosphate, pH 8.0
Flow rate:	300 cm/hr
Equilibration:	50 mM sodium phosphate, pH 8.0, 10 CV
Post-loading wash:	50 mM sodium phosphate, pH 8.0, 5 CV
Elution:	50 mM sodium acetate, pH 5.4, 15 CV

Fig. 6. Elution of mAb S monomers from a Capto adhere ImpRes Column. Elution was carried out using 50 mM sodium acetate, pH 5.4. Fractions A4 to A18 were pooled. OD 280 (—); pH (—); %B (—). The blue vertical bands represent where fractions were collected.

Summary

Aggregates of therapeutic mAbs often demonstrate different bioactivity/potency profiles, storage stability, immunogenicity, and pharmacodynamic/pharmacokinetic properties than their monomeric counterparts (Vázquez-Rey and Lang 2011). These product-related impurities must be effectively removed during the manufacturing process to ensure the safety and efficacy of the final drug formulation. A high level of aggregates was present in the mAb S harvest, which could have been an indication of the intrinsic hydrophobicity of mAb S or the exposure of its hydrophobic amino acid residues due to misfolding, unfolding, or denaturation. The mixture of monomers and aggregates of mAb S could not be separated by traditional cation exchangers (data not shown). Three mixed-mode media, CHT, Capto adhere, and Capto adhere ImpRes, were tested for their efficiency in clearing mAb S aggregates; their optimal performances are summarized in Table 3.

Table 3. Comparison of CHT, Capto adhere, and Capto adhere ImpRes in the clearance of mAb S aggregates.

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

* Step elution with 10 mM sodium phosphate and 550 mM sodium chloride, pH 7.0
** Step elution with 50 mM sodium acetate, pH 5.5
*** Step elution with 50 mM sodium acetate, pH 5.4

The contamination level of mAb S aggregates in the recovered mAb S monomer fractions was effectively reduced to below 0.5% by all three media. However, CHT offered the best monomer recovery in the smallest eluate volume (Table 3). Since the isoelectric point (pI) of mAb S was estimated at 6.9 (data not shown), it should bind to Capto adhere–based media through strong electrostatic interaction. The difficult elution of mAb S from these two hydrophobic anion exchangers suggests that a significant hydrophobic interaction was also involved in mAb S binding to these columns. A more stringent acidic condition was necessary for protonating mAb S molecules in order to promote their dissociation from these chromatography media; however, this was carried out at the expense of selectivity between monomers and aggregates. To satisfy the QC specification of ≥99.5% monomer in the final product, elution had to be terminated at pH 5.5 and pH 5.4 for Capto adhere and Capto adhere ImpRes, respectively. As a result, the recovery of mAb S monomer from these chromatography media was compromised. Tailing was observed and elution was incomplete even after 15 CV of elution buffer were applied.

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