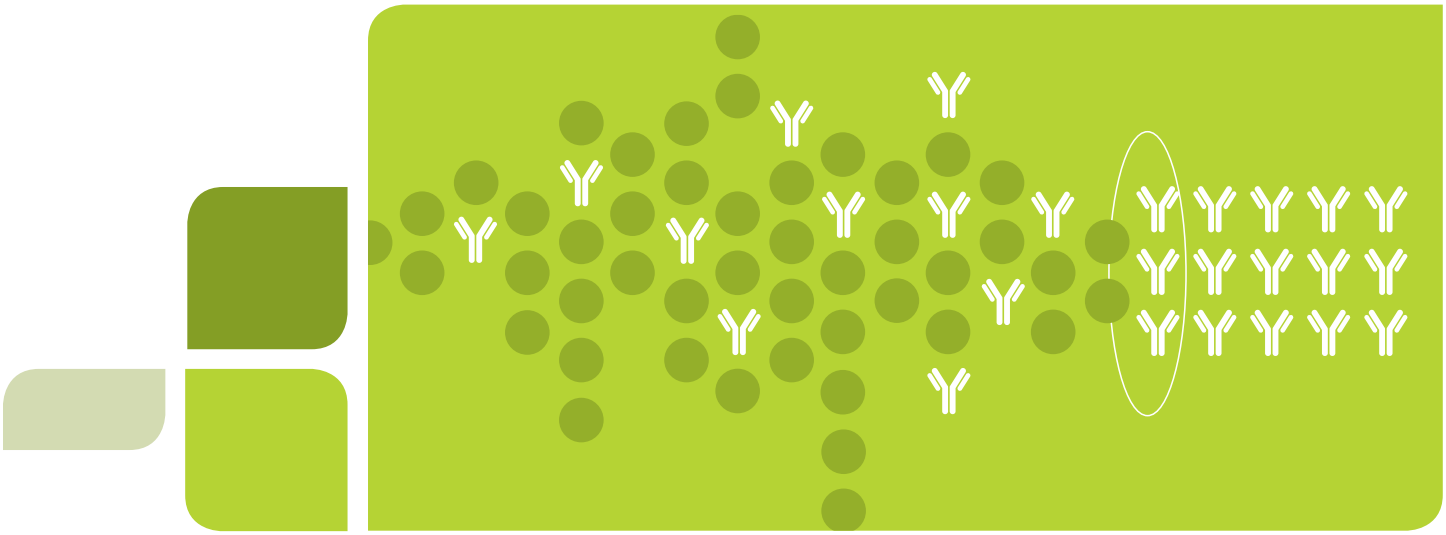

Monoclonal Antibody Purification: Polish Purification Resins

- Very closely related molecules resolved for efficient purification
- Final traces of impurities removed

Mixed-Mode Media/Resins for Monoclonal Antibody Polish Purification

Monoclonal antibody (mAb) purification processes typically involve a multistep workflow consisting of two or three steps for capture, intermediate, and polish purification. The resins selected for each of these steps must be compatible with the specific purification challenges that exist at that particular phase of purification.

Polish Purification Objectives

- Resolve very closely related molecules for efficient purification of the target molecule
- Remove final traces of impurities left over from the capture and intermediate steps

Ideal Features for Polish Purification Resins

- High resolution and recovery

Bio-Rad's Resins for mAb Polish Purification

- CHT™ Ceramic Hydroxyapatite Mixed-Mode Media
- Nuvia™ cPrime™ Mixed-Mode Resin

CAPTURE	INTERMEDIATE	POLISH
UNOsphere SUPrA™	UNOsphere™ Q	CHT Ceramic Hydroxyapatite
UNOsphere SUPrA	Nuvia™ Q	Nuvia cPrime
Nuvia™ S	Nuvia™ HR-S	Nuvia cPrime

BIO-RAD

CHT Ceramic Hydroxyapatite Mixed-Mode Media

CHT Ceramic Hydroxyapatite is the leading purification medium for today's demanding mAb process industry. It is a spherical, macroporous form of hydroxyapatite and a mixed-mode media with affinity and cation exchange chromatography capabilities (Figure 1). 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.

Bead Properties

Property	CHT Type I	CHT Type II
Function	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
Particle size	20 ± 2, 40 ± 4, 80 ± 8 µm	20 ± 2, 40 ± 4, 80 ± 8 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*
Recommended linear flow rate	50–300 cm/hr	50–300 cm/hr
Maximum operating pressure	100 bar (1,500 psi)	100 bar (1,500 psi)
Packing density (under ideal conditions)	0.63 g/ml	0.63 g/ml
Compression factor	Incompressible	Incompressible
pH stability	6.5–14	6.5–14
Shipping solution	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
Sanitization	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
Storage conditions	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
Shelf life	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.

Performance Advantages

- **Low backpressure at high flow rates** — able to withstand 100 bar (1,500 psi) pressure and offer flow rates up to 300 cm/hr
- **Superior clearance of multiple product-related impurities** — capable of clearing host cell proteins (HCPs), DNA, aggregate/dimer content, and other product- and process-related impurities to negligible levels
- **Unique selectivities** — enables the resolution of mixtures that appear homogenous with other media
- **Excellent capture at elevated flow rates** — enables processing at all scales

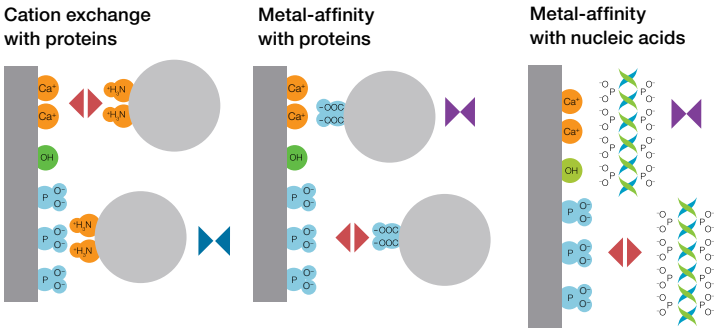

Fig. 1. Schematic representation of CHT binding mechanism. Biomolecule (●); metal affinity (⋈); electrostatic repulsion (⋈); electrostatic attraction (⋈).

Competitive Data

Best mAb monomer recovery from smallest eluate volume.
A bind and elute strategy was employed for three media: CHT (Bio-Rad Laboratories), Capto adhere (GE Healthcare), and Capto adhere ImpRes (GE Healthcare). NaCl and pH gradients were performed based on design of experiment (DoE) studies for optimized separation of aggregate and monomer. The highest total recovery was achieved at low aggregate content (≤0.5%) with CHT Media (Figure 2). CHT Media also provided the best monomer recovery for mAb S under the tested conditions (Table 1).

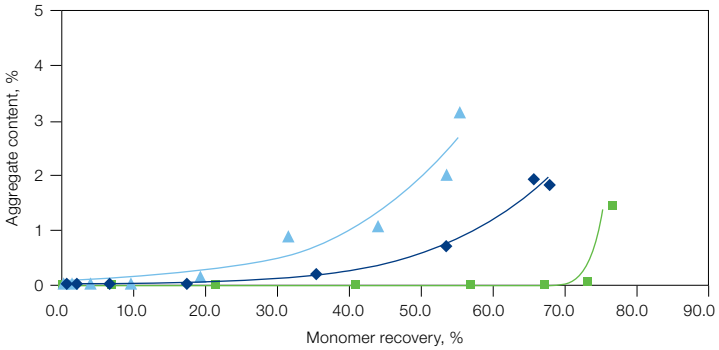

Fig. 2. Monomer recovery of mAb S. CHT Column, 1 ml, 0–1,000 mM NaCl gradient (■); Capto adhere Column, 1 ml, pH 8–5 gradient (▲); Capto adhere ImpRes Column, 1 ml, pH 8–5 gradient (◆).

Table 1. Comparison of mAb S purification performance.

Chromatography Media	Monomer Recovery, %	Eluate Volume, CV	10% DBC of mAb S, mg/ml
CHT	**83**	**5**	**47**
Capto adhere	49	14	31.9
Capto adhere ImpRes	62	14	70.9

DBC, dynamic binding capacity.

Other Resources

- CHT application guide for process development and scale-up, bulletin 6068
- Product information sheet, bulletin 5667
- CHT-based purification platform: simultaneous removal of leached Protein A, aggregates, DNA, and endotoxin from mAbs, bulletin RP0033

- CHT packing for process-scale purifications, bulletin 5739
- Chimeric mAb purification, bulletin 5853
- Separation of Fab and Fc fragments from mAb papain digest on CHT Ceramic Hydroxyapatite and CFT™ Ceramic Fluoroapatite, bulletin 5913
- Video, Learn how CHT Media can maximize purity and recovery in downstream processing, **bio-rad.com/CHTwhiteboard**
- Video, Packing CHT Media in an open process column, **bio-rad.com/CHTpacking**

Ordering Information

Catalog #	Description
Prepacked Screening Tools	
Foresight™ Columns	
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*	
732-4716	**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
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

* 2 x 96-well plates

** Package size: one row of eight columns

Nuvia cPrime Mixed-Mode Resin

The Nuvia bead is built on a polymeric base matrix that delivers low backpressure at high flow rates. It 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. It is mechanically and chemically very stable and provides unique selectivities.

Bead Properties

Property	Description
Type of ion exchanger	Hydrophobic weak cation exchange
Functional group	COO^- and NH^+
Particle size	70 ± 10 µm
Ligand density	110–150 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 (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

hIgG, human immunoglobulin G; BT, breakthrough.

Performance Advantages

- **Large design space for binding and elution** — allows for the development of highly robust methods in a commercial manufacturing setting
- **Low backpressure at high flow rates** — under 2 bar at flow rate of 600 cm/hr in DI water
- **Salt tolerant** — can be used effectively for salt- and pH-sensitive mAb purifications with minimal feed conditioning
- **Selective** — higher affinity for full length mAb relative to process impurities and by-products; ideal for the polishing step of mAb purifications
- **Flexible** — for purification of mAbs that lack an affinity handle

Data

Low backpressure at high flow rates. Nuvia cPrime is built on a porous polymeric base matrix that delivers low backpressure at high flow rates (Figure 3). Fast mass transfer dynamics ensure efficient chromatography at high flow, making Nuvia cPrime Resin an operationally superior choice for commercial scale applications.

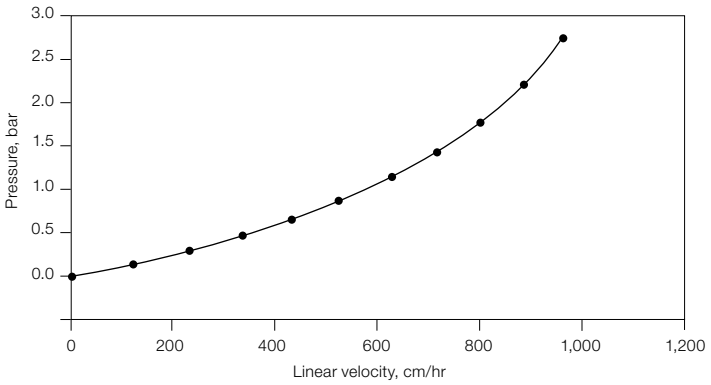

Fig. 3. Nuvia cPrime displays low backpressure at high flow rates. Flow performance of Nuvia cPrime Resin in a Bio-Rad® InPlace™ Column. A 20 x 20 cm column with 1.17 axial compression was used.

Superior binding capacity at high flow rates. Nuvia cPrime is designed for versatile capture and high recovery at high flow rates across a wide range of salt concentrations and pH (Figure 4). These capabilities, summarized in Table 2, may allow for direct loading without the need for dilution.

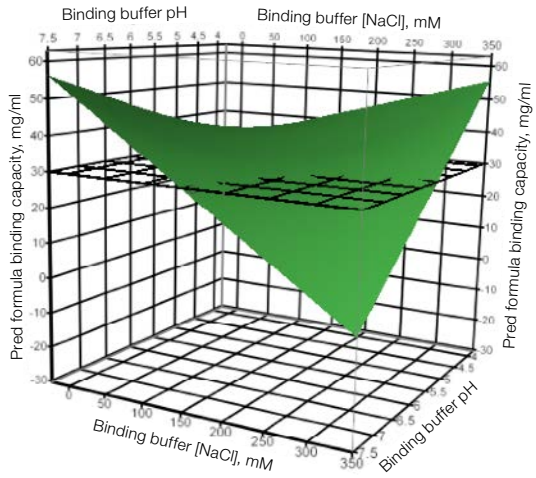

Fig. 4. Predicted binding capacity of Nuvia cPrime for mAb2 at varying pH and NaCl concentrations.

Table 2. 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%

DBC, dynamic binding capacity; BT, breakthrough.

Superior clearance of multiple product-related impurities. mAb 1 was purified with a workflow using Nuvia S for capture, Nuvia Q for the intermediate step, and Nuvia cPrime for the polish step. Nuvia cPrime was able to clear the contaminants to negligible levels (Table 3). Use of Nuvia cPrime for polishing delivers highly purified mAbs with minimal feed conditions.

Table 3. Impurity clearance.

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

Other Resources

- Instruction manual, bulletin 10023853
- Product information sheet, bulletin 6242
- Purification strategy for a clinical grade mAb using Nuvia cPrime, bulletin 6241
- A simple approach to method development (DoE) using Nuvia cPrime, bulletin 6418

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

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