

CHROMATOGRAPHY

Nuvia™ cPrime™ Hydrophobic Cation Exchange Resin

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

New Selectivity and Large Design Space for Downstream Purification Processes

Introduction

Nuvia cPrime Hydrophobic Cation Exchange Resin is a new addition to Bio-Rad's expanding family of mixed-mode purification products. Nuvia cPrime provides unique selectivity, high recovery, and versatility in large-scale purification applications for a variety of therapeutic proteins. Nuvia cPrime is effective for initial capture and polish applications, especially for molecules that present a purification challenge using current schemes.

Mixed-Mode Ligand

Nuvia cPrime Resin is designed with a mixed-mode ligand (Figure 1) 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.

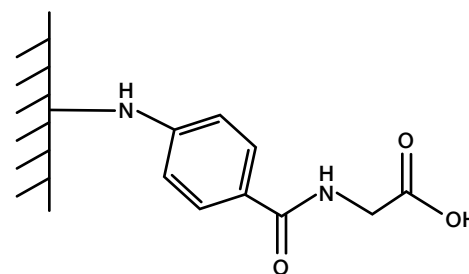


Fig. 1. Mixed-mode ligand for Nuvia cPrime Resin.

Properties of Nuvia cPrime Resin

Property	Description
Functional group	Hydrophobic weak cation exchange
Base matrix composition	Macroporous highly crosslinked polymer
Particle size	70 $\mu\text{m} \pm 10 \mu\text{m}$
Dynamic binding capacity* (hlgG)	>40 mg/ml
Dynamic binding capacity (lactoferrin)	>60 mg/ml
Ligand density	110–150 $\mu\text{eq/ml}$
Recommended linear flow rate	50–600 cm/hr
Pressure vs. flow performance**	Under 2 bar at a flow rate of 600 cm/hr
pH stability	
Short-term	3–14
Long-term	4–13
Chemical stability	1.0 N NaOH, 8 M urea, 6 M GuHCl, 6 M KSCN, 3 M NaCl, 1% Triton X-100, 2% SDS + 0.25 M NaCl, 20% EtOH, 70% EtOH, 30% IPA
Shipping solution	20% ethanol
Storage conditions	20% ethanol
Shelf life***	5 years

* At 10% breakthrough, 300 cm/hr.

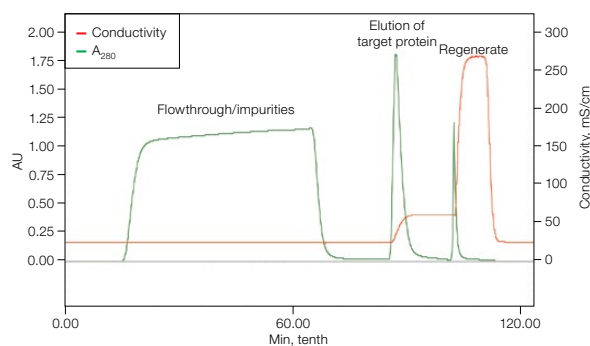
** 20 \times 20 cm packed bed (1.17 compression factor).

*** Stored at room temperature in 20% ethanol.



New and Unique Selectivity

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 to purify challenging or sensitive proteins or to separate closely related protein species, such as isoforms and variants from post-translational modifications, product aggregation, and degradation (Figure 2).



Chromatographic Condition

Condition	Specification
Column	0.56 x 4 cm
Flow rate	300 cm/hr
Loading buffer	50 mM sodium phosphate + 150 mM NaCl (pH 6.5)
Elution buffer	50 mM sodium phosphate + 400 mM NaCl (pH 7.0)
Equilibration	Loading buffer
Wash	Loading buffer
Elution	Elution buffer
Regenerate	1 N NaOH

Fig. 2. Chromatographic performance and unique selectivity. Nuvia cPrime Resin enables effective separation. AU, absorbance units.

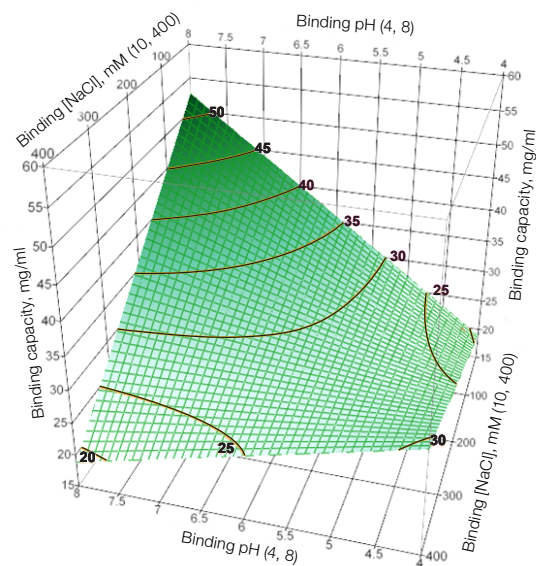
Large Design Space for Binding and Elution

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

Chromatographic Performance for Novel Therapeutics

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 now be effectively purified using simplified methods.

Lysozyme binding capacity, mg/ml



Lysozyme recovery, %

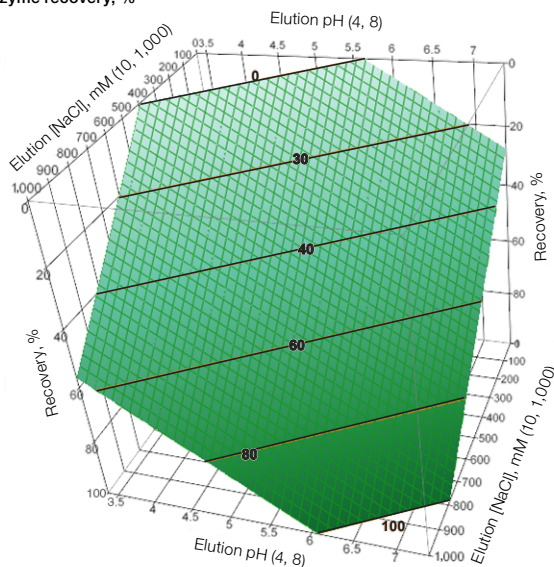


Fig. 3. Large design space afforded by Nuvia cPrime Resin.

Simple Method Development

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. Alternatively, a simple design of experiment (DOE) exercise will quickly guide developers to optimum loading, wash, and elution conditions afforded by the resin's large design space (Figures 3 and 4).

Built to Meet the Demands of Commercial Operations

Nuvia cPrime is built on a porous polymeric base matrix that delivers low backpressure at high flow rates (Figure 5). 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.

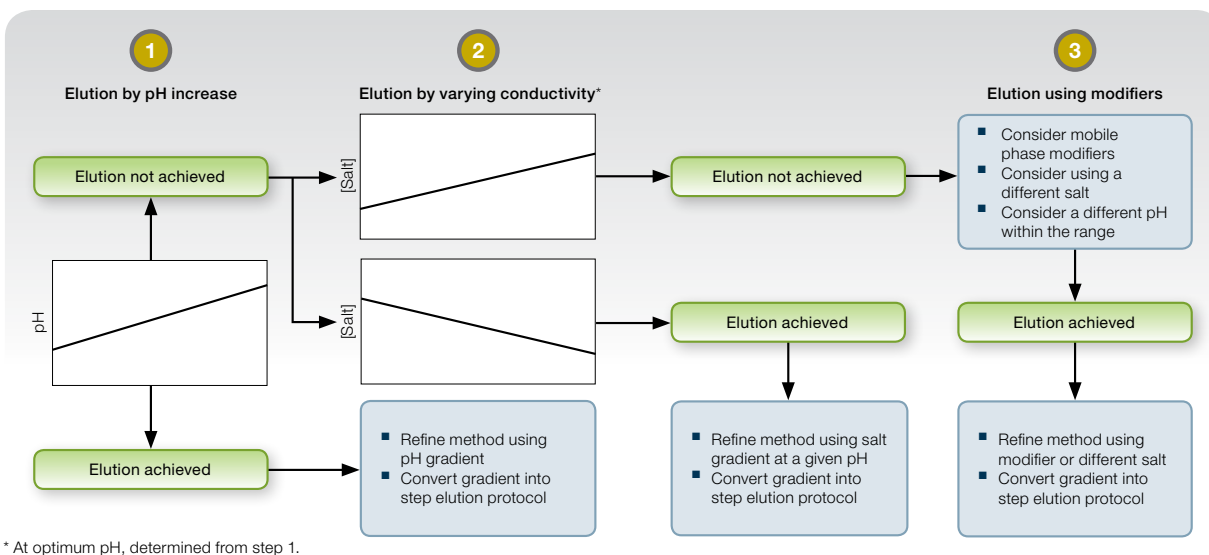
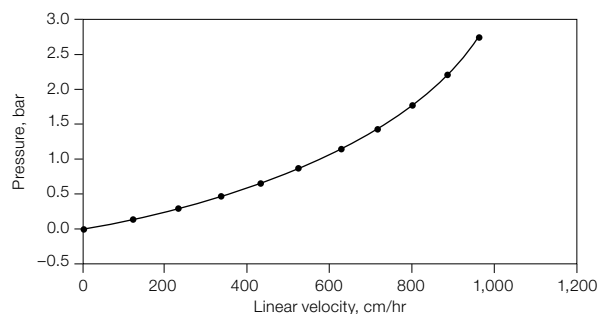


Fig. 4. Recommended approach to method development.

A. Column backpressure vs. linear velocity



B. Dynamic binding capacity vs. linear velocity, lactoferrin

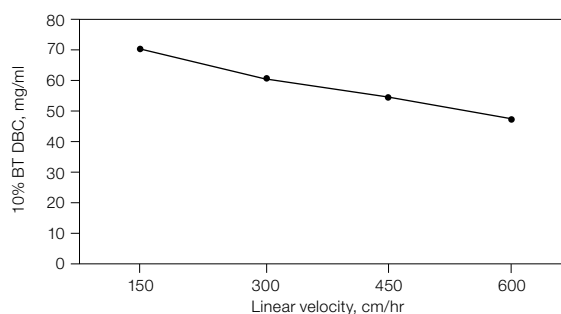


Fig. 5. Nuvia cPrime displays low backpressure at high flow rates. **A**, flow performance of Nuvia cPrime Resin in a Bio-Rad® InPlace™ Column. A 20 x 20 cm column with 17% axial compression was used. **B**, dynamic binding capacity vs. linear velocity of Nuvia cPrime Resin. A 1.1 x 9.6 cm column was loaded with 5.25 mg/ml lactoferrin in 20 mM NaOAc + 150 mM NaCl, pH 4.5, until 10% breakthrough was observed. BT, breakthrough; DBC, dynamic binding capacity.

Applications and Regulatory Support

Regulatory support files and application notes are available upon request. Bio-Rad Laboratories is an ISO 9001 registered corporation.

Please visit us at bio-rad.com/nuvia for more information about Bio-Rad's complete line of process chromatography solutions.

Ordering Information

Catalog #	Description
156-3401	Nuvia cPrime Hydrophobic Cation Exchange Resin, 25 ml
156-3402	Nuvia cPrime Hydrophobic Cation Exchange Resin, 100 ml
156-3403	Nuvia cPrime Hydrophobic Cation Exchange Resin, 500 ml
156-3404	Nuvia cPrime Hydrophobic Cation Exchange Resin, 1 L
156-3405	Nuvia cPrime Hydrophobic Cation Exchange Resin, 5 L
156-3406	Nuvia cPrime Hydrophobic Cation Exchange Resin, 10 L
732-4705	Foresight™ Nuvia™ cPrime™ Plates, 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

Larger volumes are available on request.

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