CHROMATOGRAPHY

Nuvia™ IMAC Affinity Chromatography Resin

- Easy and efficient scale-up from lab to bioprocess manufacturing scale purifications
- Optimized particle size for high flow rate and low backpressure
- Superior dynamic binding capacity at high flow rates
- Withstands repeated clean-in-place/ recharging cycles
- Excellent recovery and reproducibility
- Full regulatory support

Optimized Resin for High Productivity in Downstream Purification Processes

Introduction

Immobilized metal affinity chromatography (IMAC) is a powerful purification technique that relies on a target molecule's affinity for metal ions immobilized on polymeric particles. It offers easy, single-step removal of the majority of contaminants irrespective of their chemical properties.

Nuvia IMAC Resin is a high-capacity affinity resin built on the robust and industry-proven UNOsphere™ base bead with nitrilotriacetic acid (NTA) as the chelating ligand for di- or trivalent metal ions. It has an optimized bead size for excellent pressure/ flow properties and high dynamic binding capacity (DBC). The inert hydrophilic surface of the base beads and the chemical structure of NTA ensure highly selective binding of recombinant histidine-tagged proteins when charged with Ni^{2+} or with other transition metals, such as Zn^{2+} or Cu^{2+}.

Scalable from Laboratory to Bioprocess Manufacturing

Nuvia IMAC Resin is designed for easy scalability. It is available in small to bulk sizes to transition from bench- to pilot- to manufacturing-scale purifications. It is available in multiple user-friendly formats including Bio-Scale™ Mini Cartridges, prepacked Foresight™ Columns and Plates for purification condition screening, and bulk bottles for manufacturing-scale purifications. The optimized and narrow particle size range of Nuvia IMAC results in modest backpressure even at high flow rates. It maintains excellent reproducibility between column packing and purification runs.

High Binding Capacity at Fast Flow Rates

Nuvia IMAC Resin is designed with downstream process purification requirements in mind. Its superior mechanical strength and optimized pore surface area ensures high dynamic binding capacities even at the fast flow rates needed for process production efficiency (Figure 1). This ensures it delivers the productivity needed in downstream manufacturing.

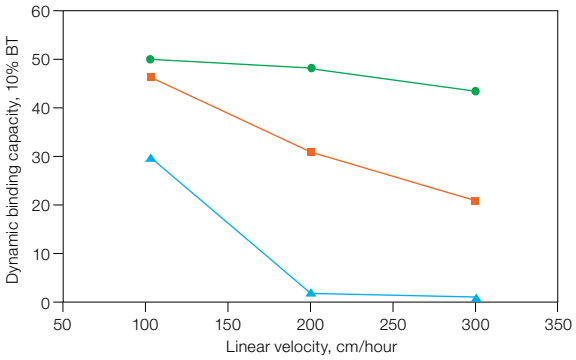

Fig. 1. Dynamic binding capacity vs. flow velocity of Nuvia IMAC Resin. Comparison of DBCs between Nuvia IMAC and two other commercially available IMACs. The resins were packed into 1 ml columns (0.5 x 5 cm). Histidine-tagged green fluorescent protein (GFP) (1.2 mg/ml) in 50 mM sodium phosphate, 5 mM imidazole, and 300 mM NaCl, pH 7.5, was loaded onto each column until 10% breakthrough (BT) was observed. Nuvia IMAC (●); agarose IMAC resin A (■); agarose IMAC resin B (▲).

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Superior Pressure/Flow Performance

Nuvia IMAC Resin is designed with an optimal bead size to achieve both laboratory- and process-scale purification of histidine-tagged proteins at high flow rates without being limited by column pressure. This leads to an increase in productivity during protein purification. The column pressure remained below 2 bar at a linear velocity of 300 cm/hr (Figure 2).

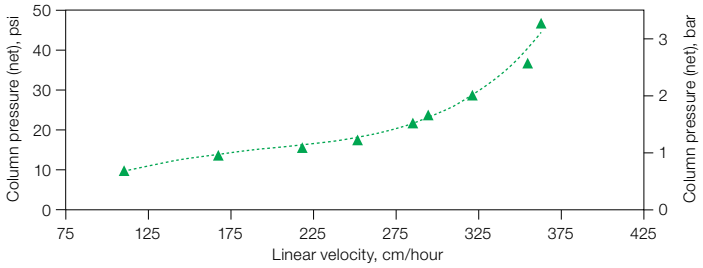

Fig. 2. Pressure/flow performance of Nuvia IMAC Resin. Uncharged Nuvia IMAC Resin slurry prepared in water was packed into a 20 x 20 cm column by axial compression with a compression factor of 1.2.

Purification of Histidine-Tagged Recombinant Protein

In addition to scalability and high DBC, Nuvia IMAC is highly selective for histidine-tagged proteins. Figure 3 shows the purification of a histidine-tagged GFP from a crude *E. coli* lysate. The various fractions shown on the chromatogram were analyzed by SDS-PAGE (Figure 4). The purity of the purified bands was estimated by densitometry to be >90%.

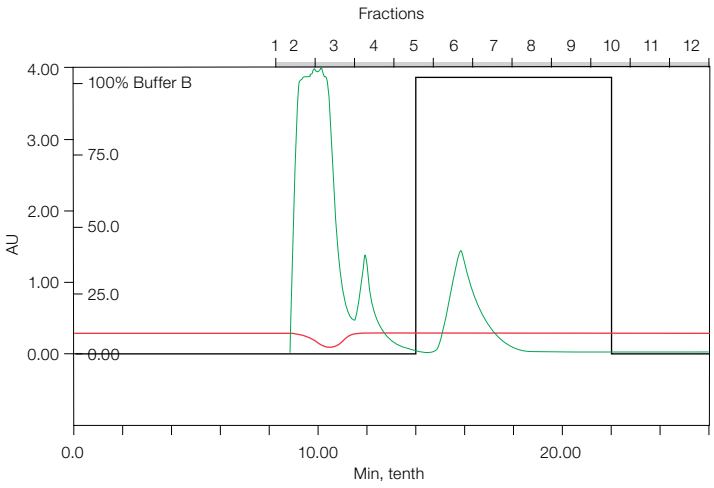

Fig. 3. Purification of histidine-tagged GFP on a Nuvia IMAC Column. *E. coli* extract (2 ml) was loaded on a 0.46 x 5 cm column in 50 mM sodium phosphate, 10 mM imidazole, and 300 mM NaCl, pH 7.5 (buffer A). The target protein was eluted with 10 column volumes (CV) 50 mM sodium phosphate, 125 mM imidazole, and 300 mM NaCl, pH 7.5 (buffer B). A_{280} (—); conductivity (—); %B (—).

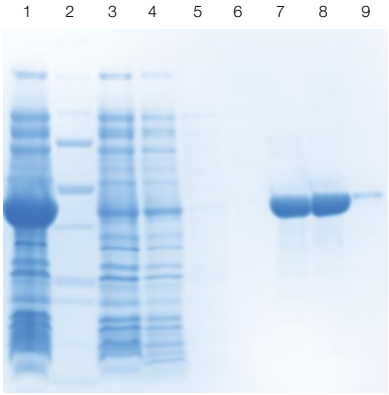

Fig. 4. Analysis of histidine-tagged GFP purified on a Nuvia IMAC Column. Fractions collected from the chromatography run shown in Figure 3 were analyzed by SDS-PAGE. 5 µl of each of the fractions were loaded onto the gel. Lane 1, *E. coli* lysate; lane 2, Precision Plus Protein™ Unstained Standards; lanes 3–9, fractions 3–9.

Excellent Recovery

Nuvia IMAC has an inert and hydrophilic base bead surface that minimizes nonspecific interaction with the beads due to charge or hydrophobicity. The pore structure of the beads is also optimized to allow efficient mass transfer under dynamic conditions. These features contribute to the excellent recovery of histidine-tagged proteins with Nuvia IMAC (Figure 5; target protein recovery is 96.8%).

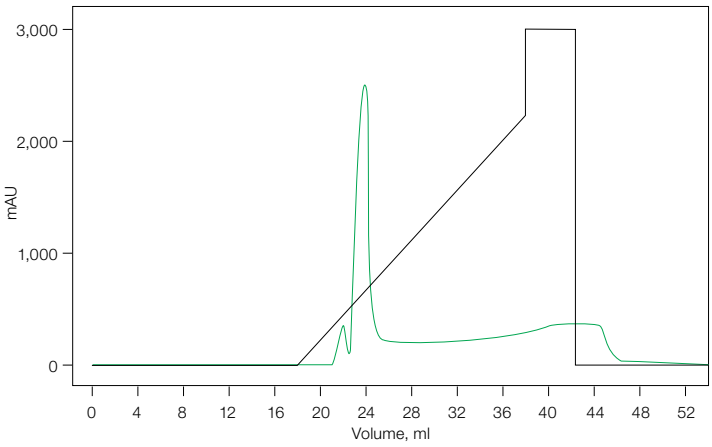

Fig. 5. Recovery of a histidine-tagged protein with Nuvia IMAC Resin. 10 mg of a 45 kD histidine-tagged protein were loaded onto a 1 ml column of Nuvia IMAC (0.46 x 5 cm) at a flow rate of 300 cm/hr. The equilibration and post-loading wash was performed with buffer A (50 mM sodium phosphate, 300 mM NaCl, pH 7.5). The elution was performed in a 20 CV linear gradient with 0–75% buffer B (50 mM sodium phosphate, 1 M imidazole, 300 mM NaCl, pH 7.5) and then held at 100% buffer B for 5 CV. Recovery of target protein was 96.8%. A_{280} (—); %B (—).

Reproducible Performance and Reusability

Nuvia IMAC Resin shows no significant loss in its DBC over more than 100 cycles of repeated use (Figure 6). It is produced by a validated manufacturing process to ensure batch-to-batch reproducibility.

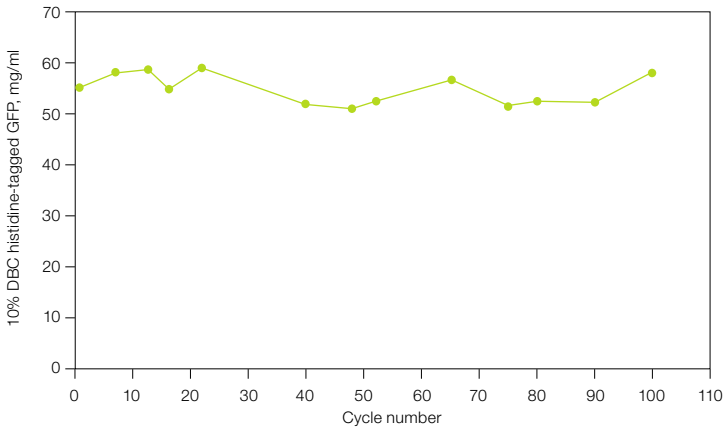

Fig. 6. Reusability of Nuvia IMAC Resin. Histidine-tagged GFP was loaded onto a 0.5 x 5.1 cm column packed with Nuvia IMAC with a compression factor of 1.25. The column was run at 300 cm/hr. The binding buffer was 50 mM sodium phosphate, 5 mM imidazole, and 300 mM NaCl, pH 7.5, and the elution buffer contained 250 mM imidazole. Solutions for column regeneration are listed in Table 1.

Wide-Range Chemical Stability and Compatibility

Nuvia IMAC Resin is stable in a broad range of chemicals (Table 1). It is compatible with all reagents traditionally used for histidine-tagged protein purification, including reducing agents, detergents, denaturing agents, and additives. The stable pH range for this resin is 2–14. For the complete list of compatible reagents and working conditions, refer to the Nuvia IMAC instruction manual (bulletin 10044307).

Table 1. Properties of Nuvia IMAC Resin.

Property	Description
Ligand	Nitrilotriacetic acid
Particle size	38–53 µm
Total ligand density	≥18 µmol/ml
Dynamic binding capacity*	>40 mg/ml at 300 cm/hr
Compression factor	1.20–1.25
Recommended linear flow rate	50–300 cm/hr
Pressure vs. flow performance	Under 2 bar at flow rate of 300 cm/hr in deionized water (20 x 20 cm packed bed, 1.2 compression factor)
pH stability	2–14
Shipping solution	2% benzyl alcohol or 20% ethanol
Regeneration	50 mM EDTA, pH 8.0 (stripping) 1 N NaOH (CIP/SIP) 0.1 M Ni_2SO_4 (recharging)
CIP solution	1 N NaOH
Sanitization	1 N NaOH
Storage conditions	20% ethanol
Chemical compatibility**	Reducing agents (β-ME, TCEP, DTT) Denaturing agents (urea, GnHCl) Detergents (Triton X-100, NP-40, CHAPS, CHAPSO) Additives (glycerol)
Chemical stability***	48 hr at 1 M NaOH 1 week at 0.01 M HCl
Shelf life	5 years

* 10% breakthrough capacity determined with 1.2 mg/ml of a 40 kD histidine-tagged protein in 50 mM sodium phosphate, 5 mM imidazole, and 300 mM NaCl, pH 7.5.

** Recharging is not required. Refer to the instruction manual for a complete list.

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Technical Assistance

We have a dedicated applications team with extensive experience in process development that is available to answer questions and provide assistance in laboratory to scale-up design. Email our process specialists at **process@bio-rad.com** for more information about this product.

Screen this resin for your application.
Visit **bio-rad.com/web/ResinSample** to request a sample.

Visit **bio-rad.com/web/ProcessResins** for more information about Bio-Rad's complete line of process chromatography resins.

Ordering Information

Catalog #	Description*
7800800	**Nuvia IMAC Resin**, 25 ml bottle, Ni-charged
7800801	**Nuvia IMAC Resin**, 100 ml bottle, Ni-charged
780-0802	**Nuvia IMAC Resin**, 500 ml bottle, Ni-charged
12004039	**Nuvia IMAC Resin**, 10 L
12004040	**Nuvia IMAC Resin**, 5 L
12004051**	**Foresight Nuvia IMAC RoboColumn Unit**, 200 µl, Ni-charged
12004052**	**Foresight Nuvia IMAC RoboColumn Unit**, 600 µl, Ni-charged
12004035***	**Foresight Nuvia IMAC Plates**, 20 µl, Ni-charged
12004038	**Foresight Nuvia IMAC Column**, 1 ml, Ni-charged
12004037	**Foresight Nuvia IMAC Column**, 5 ml, Ni-charged
7800811	**Bio-Scale Mini Nuvia IMAC Cartridge**, 1 x 5 ml column, Ni-charged
7800812	**Bio-Scale Mini Nuvia IMAC Cartridges**, 5 x 5 ml columns, Ni-charged

* Larger quantities available upon request.
** Package size: one row of eight columns.
*** Package size: 2 x 96-well plates.

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