Nuvia IMAC Resin

Instruction Manual

Catalog numbers

7800800
7800801
780-0802
12002782
12003233
12018135
12018162
12004039
12004040

Please read these instructions prior to using Nuvia IMAC Resins. If you have any questions or comments regarding these instructions, contact your Bio-Rad Laboratories representative.

BIO-RAD

Table of Contents

Section 1
Introduction

Immobilized metal affinity chromatography (IMAC) is a powerful purification technique that relies on a biomolecule's affinity for metals immobilized onto a chelating surface. The chelating ligand may be charged with transition metals such as Ni^{2+}, Cu^{2+}, Co^{2+}, or Zn^{2+}. Proteins tagged with histidine or naturally rich in histidine/cysteine residues bind with high selectivity to the metal ions and are then strongly retained on porous chromatographic supports. The strong affinity of the molecule for metal ions often makes extensive optimization unnecessary.

Nuvia IMAC Resin is optimized for high productivity in downstream purifications. It is compatible with high flow rates and offers superior binding capacity. It can be easily scaled up from lab- to bioprocess-scale manufacturing. Refer to the Nuvia IMAC product information sheet (bulletin 6859) and Tables 1, 2, and 3 for more product details.

If you have questions or require method development assistance with Nuvia IMAC Resin, please contact your local Bio-Rad process chromatography representative or the Bio-Rad technical support group for assistance at 1-800-4-BIORAD (1-800-424-6723).

Section 2
Product Description

Nuvia IMAC Resin is based on Bio-Rad's innovative UNOsphere Beads, which are manufactured using proprietary polymerization and derivatization technologies, with nitrilotriacetic acid (NTA) as the functional ligand. The tertiary amine and carboxylic acid side chains of NTA serve as the chelating groups for divalent metal ions.

Nuvia IMAC Resin is provided as 50% (v/v) slurry in 20% ethanol or 2% benzyl alcohol. Multiple pack sizes are available for process- and laboratory-scale use, in both uncharged and Ni^{2+} charged versions. Multiple formats are available, including Bio-Scale Mini Cartridges and prepacked Foresight Columns and Plates for purification condition screening, and bottles for manufacturing-scale purifications.

The technical specifications of Nuvia IMAC Resin are listed in Table 1; chemical compatibility and stability are shown in Tables 2 and 3.

Table 1. Characteristics of Nuvia IMAC Resins.

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 buffer (20 x 20 cm packed bed, 1.2 compression factor)
pH stability	2–14
Shipping solution	20% ethanol or 2% benzyl alcohol
Regeneration	50 mM EDTA, pH 8.0 (stripping) 1 N NaOH (CIP/SIP) 100 mM Ni_2SO_4 (recharging)
CIP solution	1 N NaOH
Sanitization	1 N NaOH
Storage conditions	20% ethanol or 2% benzyl alcohol
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, using a 1 ml (0.5 x 5 cm) column. EDTA, ethylenediaminetetraacetic acid; CIP, cleaning-in-place; SIP, sanitization-in-place.

Table 2. Chemical compatibility.

Reducing agents*	**Compatibility concentration**
DTE	10 mM
DTT	10 mM
ß-Mercaptoethanol	20 mM
TCEP	20 mM
Reduced glutathione	20 mM
Denaturing agents	
Guanidine hydrochloride	6 M
Urea	8 M
Detergents	
Triton X-100 (nonionic)	5%
Tween 20 (nonionic)	5%
NP-40 (nonionic)	5%
Cholate (anionic)	5%
SDS (anionic)	5%
Sodium lauroyl sarcosinate (anionic)	2%
Cetyltrimethylammonium bromide (CTAB) (cationic)	5%
CHAPS (zwitterionic)	5%
CHAPSO (zwitterionic)	5%
Additives	
Glycerol	50%
Na_2SO_4	200 mM
NaCl	2 M
EDTA	2 mM**
EGTA	10 mM
Na citrate	100 mM
Imidazole	500 mM
Ethanol	20%
Ca^{2+}	10 mM***
Mg^{2+}	100 mM***
Amino acids	Not recommended
$(NH_4)_2\ SO_4$	2 M†

Table 2. Chemical compatibility, continued.

Buffer substances	Compatibility concentration
NaPi, pH 7.5	100 mM
KPi, pH 7.5	100 mM
HEPES, pH 7.5	100 mM
MOPS, pH 7.5	100 mM
Tris HCl, pH 7.5	100 mM
Buffer pH range	~7–8

* For best results, perform a blank run before loading by washing the column with 5 column volumes (CV) of buffer, 5 CV of elution buffer, and then equilibrating the column with ~5–10 CV of binding buffer.

** Static binding capacity for a 45 kD protein ≥20 mg per ml of resin.

*** HEPES or Tris buffers should be used to prevent precipitation.

† Higher concentrations may cause protein precipitation.

Table 3. Chemical stability. The treatments have no effect on binding.

Chemical	Treatment
500 mM imidazole*	2 hr, RT, re-equilibration
5 mM DTT*	24 hr, RT, re-equilibration
5 mM TCEP*	24 hr, RT, re-equilibration
20 mM β-ME*	24 hr, RT, re-equilibration
6 M GnHCl*	24 hr, RT, re-equilibration
10 mM EDTA**	72 hr, RT, recharge, re-equilibration
100 mM EDTA**	2 hr, RT, recharge, re-equilibration
100 mM HOAc*	72 hr, RT, re-equilibration
10 mM HCl**	1 week, RT, recharge, re-equilibration
100 mM NaOH*	1 week, RT, re-equilibration
1 M NaOH*	48 hr, RT, re-equilibration

RT, room temperature.
* No recharging is needed.
** Requires recharging.

Section 3
General IMAC Procedures

Nuvia IMAC Resin is provided fully hydrated as a 50% (v/v) slurry in 20% ethanol or 2% benzyl alcohol. For column packing, replacing the shipping solution with packing buffer is recommended. Small volumes of Nuvia IMAC Resin can be easily washed in a Büchner funnel with 4–5 bed volumes of buffer. For large volume preparation, cycle through 3–4 settling and decanting steps using buffer.

Low concentrations (1–15 mM) of imidazole are recommended in equilibration buffer, which will aid in reducing nonspecific binding of weakly interacting contaminant proteins. Imidazole levels should be optimized for each protein based on the concentrations required to prevent nonspecific binding while enabling target protein adsorption.

Protein Binding

Optimal protein binding with Nuvia IMAC Resin is achieved at pH 7.0–8.0. Proteins containing engineered histidine tags, as well as untagged proteins rich in histidine and/or cysteine residues, can bind to Nuvia IMAC Resin with varying affinities.

Recommended equilibration and binding buffer: 20–50 mM sodium or potassium phosphate, containing up to 1 M NaCl to reduce nonspecific protein binding

Washing

The optimal pH and/or imidazole concentration to be used in wash buffers is protein dependent and should be determined experimentally.

Recommended wash buffer: 5–30 mM imidazole, 50 mM sodium phosphate, and 300 mM NaCl

Elution

Proteins can be eluted with higher concentrations of imidazole in the elution buffer.

- Recommended elution buffer:
  - 5–500 mM imidazole, 50 mM sodium phosphate, and 300 mM NaCl

- Histidine-tagged proteins can also be eluted by:
  - Introduction of a competitor ligand in a step or gradient elution with ligands such as histidine or glycine
  - Reduction of the pH to 4.5–5.3
  - Stripping of the immobilized metal with chelating agents such as EDTA or EGTA

Section 4
Lab-Scale Column Packing

Medium-Pressure Columns

Slurry packing is preferred for small columns. For best results, use 5–50 mm inner diameter (ID) columns and a bed height of 5–30 cm.

Recommended Columns

Bio-Rad's Bio-Scale MT High-Resolution Columns may be used. They are convenient to use with Bio-Rad's NGC Chromatography System or any medium- or high-pressure system:

- 7510081 — Bio-Scale MT2 Column (7 x 52 mm) for bed volumes up to 2 ml
- 7510083 — Bio-Scale MT5 Column (10 x 64 mm) for bed volumes up to 5 ml
- 7510085 — Bio-Scale MT10 Column (12 x 88 mm) for bed volumes up to 10 ml
- 7510087 — Bio-Scale MT20 Column (15 x 113 mm) for bed volumes up to 20 ml

Materials

- Empty column (1–5 cm ID x 30 cm) with flow adaptors, inlet and outlet ports, glass filter, Nuvia IMAC Resin, packing reservoir, pump

Resin Preparation

Nuvia IMAC Resins are supplied in a 20% ethanol or 2% benzyl alcohol solution. Small columns can be packed in this solution, although removal of storage solution is preferred. Before applying sample, ensure that all the storage solution is removed during the equilibration step. Ni-charged Nuvia IMAC Resin is ready to use. The uncharged resin can be charged according to the protocol mentioned in Section 7.

Method

1. Eliminate air from the column dead spaces. Attach the inlet of a peristaltic or other pump to the outlet of the column. Fill the column with buffer to about 10% of its volume. Flush end pieces with buffer to ensure that the bottom of the bed support is fully saturated and free of air bubbles. Allow a few centimeters of buffer to remain when closing the outlet valve.
2. Suspend the resin in a beaker by gently swirling or stirring with a glass or plastic rod.
3. Carefully transfer about a third of the slurry down the side into the column using a glass or plastic rod to avoid introducing air bubbles.
4. Start the pump at a low flow rate (for example, 0.5 ml/min). The resin will begin to pack in the column. As the liquid level in the column drops, continue to transfer the rest of the slurry until the packed bed reaches 1 cm from the top. Stop or slow the pump flow rate as necessary.
5. Gently add more buffer down the side of the column to make sure the liquid does not fall below the resin level. Continue adding buffer until the bed seems to have stabilized. Then gently fill the column to the top with buffer.
6. Stop the pump.
7. Insert the adaptor into the column at an angle to avoid introducing any air bubbles. Make sure the exit tubing is open so that buffer can flow out the top adaptor along with any air.
8. Adjust the adaptor to sit directly on top of the resin bed.
9. Open the column outlet and pump buffer through the column at a) a packing flow rate of ~400–600 cm/hr for 5–10 min, or b) the maximum pressure allowed by the column hardware and resin. The resin bed will compress while packing at high flow rates. Mark the compression level with a pen.
10. Stop the flow. At this point, the resin bed height may readjust and rise. If this happens, adjust the flow adaptor to compress the bed another 0.1–0.5 cm past the level marked with the pen.

11. Reconnect the pump and equilibrate. Pass eluent (equilibration buffer) through the column at the packing flow rate. During equilibration, the bed may compress even further. When a constant bed height is reached, mark the compression level at this flow rate. Again, adjust the adaptor to compress the bed an additional 0.1–0.5 cm past the level marked.

Note: Chromatographic steps during purification should not be run at greater than 75% of the packing flow rate.

Section 5
Process-Scale Column Packing

10 x 20 cm Column

The packing method example shown in this section was done on a 10 x 20 cm BPG Column (Cytiva).

1. Remove the air in the bottom frit of the column with buffer and then close the bottom outlet.
2. Calculate the amount of resin required to pack the column at the desired bed height based on the slurry concentration and compression factor. Pour the resin slurry (concentration 45–65%) into the column.
3. Allow the resin to settle for 30 min to have a liquid gap of 2–3 cm above the bed.
4. Lower the top adaptor to 1 cm below the liquid surface. Gently shake the adaptor to remove the air near the seal and underneath the adaptor.
5. Tighten the seal and lower the adaptor slightly to push out the remaining air in the adaptor through the waste line of the top valve.
6. Connect the pump to the top valve and open the bottom valve.
7. Consolidate the bed by pumping buffer through the column at 60 cm/hr until a stable bed is formed.
8. Stop the pump and close the bottom outlet.
9. Wait ~10 min to allow the bed to settle completely.
10. Record the settled bed height and calculate the settled bed volume to verify the calculation of resin volume.
11. Loosen the seal slightly and lower the top adaptor to 1 cm above the settled bed surface. Tighten the seal well.
12. Calculate and mark the target bed height based on the target compression factor.
13. Close the top adaptor outlet and open the bottom outlet.
14. Push down the top adaptor slowly (approximately 100 cm/hr) to compress the bed to the target bed height.
15. Condition the column with 3 CV of buffer at downward flow of 150 cm/hr.

20 x 20 cm Column

The packing method example shown in this section was performed in a 20 x 20 InPlace Column, following removal of bulk ethanol by 3–4 successive decantations.

1. Remove the air in the bottom frit of the column with buffer and then close the bottom valve.
2. Calculate the amount of resin required to pack the column at the desired bed height based on the slurry concentration and the compression factor. Pour the resin slurry (concentration 45–65%) into the column.
3. Allow the resin to settle for 30 min to have a liquid gap of 2–3 cm above the bed.
4. Lower the top piston to approximately 1 cm below the liquid surface. Gently shake the piston to remove the air around the seal and underneath the piston.
5. Inflate the seal to 4 bars and lower the piston slightly to push out the remaining air inside the piston through the waste line of the top valve.
6. Close the top valve and open the bottom valve.
7. Set the piston speed to 200 cm/hr.
8. Set the target bed height.
9. Start the axial compression.
10. Monitor the piston position during compression to make sure it stops at the target bed height. Reset the bed height as needed.
11. Close the bottom valve when the compression completes.
12. Inflate the seal to 6 bars.
13. Condition the column with 3 CV of buffer at downward flow of 150 cm/hr.

Section 6
Evaluation of Column Packing

Poor column packing can lead to compromised product quality and economics. Therefore the efficiency of packing must be tested after each column packing. In addition, packing analysis during process development can assist in setting appropriate acceptance criteria during scale-up.

After column packing is complete, equilibrate the column with up to 5 CV of equilibration buffer. To test the efficiency of the column packing operation, inject a sample of a low molecular weight, unretained compound (for example, acetone or 1 M NaCl) to determine the height equivalent to a theoretical plate (HETP). The recommended sample volume is 1–2% of the total column volume, with a linear flow rate of 100–150 cm/hr. To obtain comparable HETP values among columns, the same conditions must be applied. Suggested minimum theoretical plate values should be 1,000–3,000 plates/m but wherever possible should be based on conditions used during process development.

HETP = L/N
where
L = Bed height (cm)
N = Number of theoretical plates
Calculation for $N = 5.54(V_e/W_{½h})^2$
where
V_e = Peak elution volume or time
$W_{½h}$ = Peak width at peak half height in volume or time
V_e and $W_{½h}$ should always be in the same units
Reduced plate height can also be used to evaluate column packing efficiency.

The reduced plate height h is calculated as follows:
h = HETP/d where d is the diameter of the beads

Peak asymmetry factor calculation:
A_s = b/a
a = Front section of peak width at 10% of peak height bisected by line denoting V_e
b = Back section of peak width at 10% of peak height

Section 7
Immobilizing Metal Ions

Protein selectivity may be optimized through the choice of metal ion used. Uncharged Nuvia IMAC Resin can be used to select the best metal ion targeted to a specific protein. The protocol for immobilizing a metal ion is shown below; for initial screening, typically Ni^{2+} or Cu^{2+} is chosen:

1. After column packing is complete, the column is ready for the removal or addition of metal ions.
2. If necessary, strip any metal ion by washing with 10 CV of 50 mM sodium phosphate, 300 mM NaCl, and 50 mM EDTA at pH 8.0. Any color due to the presence of metal ions should be eliminated by this step.
3. Equilibrate the column with 5 CV of 50 mM sodium acetate at pH 4.5.
4. Prepare a 100 mM solution of the metal ion of choice. For best results, the pH of the solution should be <7.
5. Apply 3 CV of the metal ion solution.
6. Wash with 5 CV of 50 mM sodium acetate at pH 4.5.
7. Wash with 10 CV of buffer.
8. Equilibrate, if necessary, with at least 5 CV of starting buffer for the purification.

Note: Uncharged resin will be white; charged areas will be colored.

Section 8
Sample Preparation, Purification, and Optimization

Preparation

The sample should be free of particulate matter prior to application. This can be achieved by centrifugation or filtration. The choice of binding buffer will vary based on the sample properties. Sodium or potassium phosphate is recommended as a general starting buffer. The choice of elution buffer will vary depending on the procedure used. For example, a range of imidazole concentrations (5–500 mM) may be used to elute bound protein from Nuvia IMAC Resin. As an alternative to imidazole elution, the pH can be lowered such that the chelating residues, most often histidine, are protonated. This can often be accomplished by decreasing buffer pH to 5.0 or lower but should be determined experimentally. (**Note:** Below pH 4, metal ions will be stripped off the resin.) Chelating agents such as EGTA or EDTA can also be used to dissociate the protein from the resin. (**Note:** With chelating agent, metal ions will be stripped off with the protein.) Suggested buffers are shown below.

Binding/wash buffer: 50 mM sodium phosphate at pH 8.0 with 300 mM NaCl and 5–30 mM imidazole. If protein binding is weak, reduce the concentration of imidazole

Elution buffer: 50 mM sodium phosphate at pH 8.0 with 300 mM NaCl and 5–500 mM imidazole

Purification Protocol for a Packed Column

1. Equilibrate the column with at least 3 CV of binding buffer.
2. Apply the sample onto the column using the desired flow rate.
3. Wash the resin with at least 3 CV of wash buffer to remove unbound samples or until the absorbance at 280 nm is at or near baseline.
4. Collect fractions, if desired, from the wash step (unbound proteins).
5. Elute bound proteins either using a step change in buffer or as a linear gradient.
6. Collect fractions, if desired, from the elution step (bound proteins).
7. The collected fractions can be further analyzed using absorbance at A_{280}, SDS-PAGE, ELISA, etc.

Purification Protocol for a Spin Column

Part 1. Binding the sample

1. Place the prepacked spin column in an appropriate spin collection tube.
2. Pre-equilibrate the spin column with 5 CV of binding buffer.
3. Add an appropriate amount of the sample (≤0.5 ml) to the micro spin column.
4. Mix by pipetting up and down 5 times. Incubate for up to 30 min in micro spin column.
5. Centrifuge at 1,000 × g for 1 min to remove the unbound proteins.

Part 2. Washing the sample

1. Insert the micro spin column into a new, clean collection vessel.
2. Wash the resin with at least 5 CV of wash buffer. Pipet up and down at least 5 times.
3. Centrifuge at 1,000 × g for 1 min to remove remaining unbound proteins. The wash step can be repeated if necessary.

Part 3. Eluting the sample

1. Insert the micro spin column into a new, clean collection vessel.
2. Elute bound proteins with 5 CV of elution buffer. Pipet up and down at least 5 times and incubate for up to 5 min.
3. Centrifuge at 1,000 × g for 1 min to remove bound proteins.
4. Analyze fractions from above steps using absorbance at A_{280}, SDS-PAGE, ELISA, etc.

Optimization

For optimal protein purification, it is crucial that the imidazole concentration in the sample and the binding, elution, and wash buffers be empirically established. Gradient elution tests using a gradient mixer coupled to a chromatography system, such as the NGC System, can be used to optimize imidazole concentrations.

- For the binding buffer, the concentration of imidazole can be started at 5 mM. If large amounts of contaminants are also adsorbed onto the resin, the concentration of imidazole in the sample and equilibration buffer may be increased until the target protein begins to flow through. The imidazole concentration should then be reduced to below this value. This will optimize the column's binding capacity for the target protein due to the reduction in contaminating proteins
- For the wash step, use an imidazole concentration slightly lower than the concentration necessary to elute the target protein. This will increase purity by removing bound contaminants without eluting the target proteins
- The elution buffer should contain imidazole corresponding to the concentration required to elute the target protein

Section 9
Regenerating, Cleaning, Sanitizing, and Storing

Nuvia IMAC Resin should be cleaned prior to reuse. The extent of cleaning depends on the downstream application.

Regeneration

Regenerate metal-charged Nuvia IMAC Resin by first stripping with an EDTA solution. Wash the column with 10 CV of 50 mM sodium phosphate, 300 mM NaCl, and 50 mM EDTA at pH 8.0. Remove residual EDTA from the column by washing with 3–5 CV of binding buffer. Equilibrate the column with 5 CV of 50 mM sodium acetate at pH 4.5. Recharge with the metal ions as described in Section 7.

Cleaning in Place

Wash the column with the following solution.

- 0.1 N NaOH up to 3 hr (removes precipitated, hydrophobic, and lipoproteins)

Alternatively, cleaning-in-place solutions such as NaCl, which removes ionic contaminants, or other solutions that solubilize precipitated, hydrophobic, and lipoproteins can can be used.

Sanitization

The column may be sanitized with 1 N NaOH.

Storage

Nuvia IMAC Resin is stable at room temperature across a broad pH range (2–14). The resin may also be stored in either of the following solutions:

- 2% benzyl alcohol
- 20% ethanol

Uncharged resin can be stored with 0.1 N NaOH.

Section 10
Regulatory Support

A regulatory support file is available for Nuvia IMAC Resin. If you need assistance validating the use of Nuvia IMAC Resin in a production process, contact your local Bio-Rad representative.

Section 11
Troubleshooting Guide

Possible Cause	Solution
Sample is too viscous	
High concentration of host nucleic acids in lysate	Viscosity of extract can be reduced by nuclease treatment
Sample application causes column to clog	
Insufficient clarification of sample	Prevent cell debris from clogging the column by increasing the centrifugation speed and/or filtering the sample
Protein precipitation	Consider the use of additives to improve protein stability and solubility
No protein is eluted	
Target protein is in the flowthrough	Reduce imidazole concentration in sample and binding/wash buffers. An imidazole gradient may be used to determine optimal concentrations for wash and elution conditions
	Check pH levels of sample. Adjust pH to 7–8
	The histidine tag may not be accessible
	Use denaturing conditions to purify protein or reclone the plasmid construct with the histidine-tagged sequence placed at the opposite terminus
	Proteolytic cleavage during fermentation or purification may have caused the histidine tag to be removed. Add protease inhibitors or make a new construct with histidine tag attached to other terminus
Elution conditions are too mild or protein may be in an aggregated or multimer form	Increase the concentration of imidazole in elution buffer; optimize elution buffer pH
Protein precipitates during purification	
Aggregate forms	Add solubilization agents to samples and/or buffers: 0.1% Triton X-100, Tween 20, 20 mM β-mercaptoethanol, and ≤20% glycerol to improve protein solubility
Poor recovery of target protein	
Protein is in the flowthrough	See recommendations in No protein is eluted section, above
Binding capacity of the column has been exceeded	Increase the column size or reduce the sample volume
Strong adsorption of the target protein to the matrix	Reduce hydrophobic adsorption by including detergents or organic solvents, or by increasing the concentration of imidazole and/or NaCl

Possible Cause	Solution
Histidine-tagged protein is not pure	
Contaminants elute with target protein	Make binding and wash steps more stringent. Include 10–20 mM imidazole in binding and wash buffers
	Prolong the imidazole-containing wash step
	Column is too large; reduce amount of Nuvia IMAC Resin used
Strongly bound contaminants elute with protein	Very high concentrations of imidazole will cause strongly bound contaminants to elute as well. Reduce the imidazole concentration during the elution
Association of contaminating proteins with target protein via disulfide bonds	Include ≤20 mM β-mercaptoethanol
	Note: Exercise caution if using DTT
Association between the histidine-tagged protein and protein contaminant	Add nonionic detergent or alcohol (that is, Triton X-100, Tween 20, or glycerol) to reduce hydrophobic interactions. Concentration of NaCl may be increased to minimize electrostatic interactions

Section 12
Ordering Information

Catalog #	Description
Bottles*	
7800800	**Nuvia IMAC Ni-Charged Resin**, 25 ml
7800801	**Nuvia IMAC Ni-Charged Resin**, 100 ml
780-0802	**Nuvia IMAC Ni-Charged Resin**, 500 ml
12002782	**Nuvia IMAC Ni-Charged Resin**, 10 L
12003233	**Nuvia IMAC Ni-Charged Resin**, 5 L
12018135	**Nuvia IMAC Uncharged Resin**, 25 ml
12018162	**Nuvia IMAC Uncharged Resin**, 500 ml
12004039	**Nuvia IMAC Uncharged Resin**, 10 L
12004040	**Nuvia IMAC Uncharged Resin**, 5 L
Prepackaged Formats	
12004051**	**Foresight Nuvia IMAC Ni-Charged RoboColumn Unit**, 200 µl
12004052**	**Foresight Nuvia IMAC Ni-Charged RoboColumn Unit**, 600 µl
12004035***	**Foresight Nuvia IMAC Ni-Charged Plates**, 20 µl
12004038	**Foresight Nuvia IMAC Ni-Charged Column**, 1 ml
12004037	**Foresight Nuvia IMAC Ni-Charged Column**, 5 ml
12009289****	**EconoFit Nuvia IMAC Uncharged Column**, 1 x 1 ml, 7 x 25 mm

* Larger quantities available upon request.
** Package size: 1 row of 8 columns.
*** Package size: 2 x 96-well plates.
**** Maximum pressure 45 psi.

Section 13
Bibliography

Block H et al. (2009). Immobilized-metal affinity chromatography (IMAC): A review. Methods Enzymol 463, 439–473.

Hochuli E (1988). Large-scale chromatography of recombinant proteins. J Chromatogr 444, 293–302.

Porath J et al. (1975). Metal chelate affinity chromatography, a new approach to protein fractionation. Nature 258, 598–599.

Bio-Rad Laboratories, Inc.

Life Science Group

Website *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800*
Austria *00 800 00 24 67 23* ***Belgium*** *00 800 00 24 67 23* ***Brazil*** *4003 0399*
Canada *1 905 364 3435* ***China*** *86 21 6169 8500*
Czech Republic *00 800 00 24 67 23* ***Denmark*** *00 800 00 24 67 23*
Finland *00 800 00 24 67 23* ***France*** *00 800 00 24 67 23*
Germany *00 800 00 24 67 23* ***Hong Kong*** *852 2789 3300*
Hungary *00 800 00 24 67 23* ***India*** *91 124 4029300* ***Israel*** *0 3 9636050*
Italy *00 800 00 24 67 23* ***Japan*** *81 3 6361 7000* ***Korea*** *82 080 007 7373*
Luxembourg *00 800 00 24 67 23* ***Mexico*** *52 555 488 7670*
The Netherlands *00 800 00 24 67 23* ***New Zealand*** *64 9 415 2280*
Norway *00 800 00 24 67 23* ***Poland*** *00 800 00 24 67 23*
Portugal *00 800 00 24 67 23* ***Russian Federation*** *00 800 00 24 67 23*
Singapore *65 6415 3188* ***South Africa*** *00 800 00 24 67 23*
Spain *00 800 00 24 67 23* ***Sweden*** *00 800 00 24 67 23*
Switzerland *00 800 00 24 67 23* ***Taiwan*** *886 2 2578 7189*
Thailand *66 2 651 8311* ***United Arab Emirates*** *36 1 459 6150*
United Kingdom *00 800 00 24 67 23*

D116698 Ver D US/EG *23-0551 0723 Sig 0123*