Nuvia™ IMAC Resin

Instruction Manual

Catalog numbers

7800800
7800801
7800802
12003233
12002782
12004040
12004039

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 (U.S. patent 6,423,666), 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. The multiple user-friendly formats include 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 deionized water (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

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 CV of DI water, 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, recharge, re-equilibration
1 M NaOH**	48 hr, RT, recharge, re-equilibration

* No recharging is needed
** Requires recharging

RT, room temperature

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 water or buffer. For large volume preparation, cycle through 3–4 settling and decanting steps using water or buffer.

Low concentrations (0.001–15 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:
  - 30–500 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, histamine, 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 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 distilled water to about 10% of its volume. Flush end pieces with distilled water to ensure that the bottom of the bed support is fully saturated and free of air bubbles. Allow a few centimeters of distilled water 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 distilled water down the side of the column to make sure the liquid does not fall below the resin level. Continue adding distilled water until the bed seems to have stabilized. Then gently fill the column to the top with distilled water.
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 distilled water 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 distilled 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 (distilled water or 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.

Sample Preparation–Size Columns

Equipment

- Sample preparation–size columns (for example, Micro Bio-Spin™ Columns, catalog #7326207), plasticware, 2 ml capped and 2 ml capless tubes, Nuvia IMAC Resin, tabletop centrifuge, 1 ml pipet with wide-bore pipet tips

Method

1. Thoroughly suspend Nuvia IMAC Resin.
2. Place the column into an appropriate collection vessel, for example, a 2 ml capless collection tube.
3. Using a pipet, transfer the appropriate amount of Nuvia IMAC Resin to a microcentrifuge tube. If using a Micro Bio-Spin Column, transfer 0.2 ml slurried 50% v/v Nuvia IMAC Resin to the column. This is equivalent to ~100 µl of a packed resin bed.
4. Centrifuge at 1,000 x g for 15 sec to pack resin and remove storage solution.
5. Wash column with at least 5 column volumes (CV), or ~500 µl, of distilled water. Centrifuge at 1,000 x g for 15 sec to pack resin.

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.

1. Remove the air in the bottom frit of the column with water 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 water through the column at 60 cm/h 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/h) to compress the bed to the target bed height.
15. Condition the column with 2 CV of water at downward flow of 320 cm/h.

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 water 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/h.
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 2 CV of water at downward flow of 200 cm/h and 23 psi.

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 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). If acetone is used as the test marker (use an ultraviolet absorbance monitor set at 280 nm), the equilibration buffer must have a salt concentration <100 mM. If 1 M NaCl is the test marker (use a conductivity monitor), then the equilibration buffer salt concentration should be 100–200 mM. The recommended sample volume is 1–2% of the total column volume. Column testing should be operated using the same linear velocity used to load and/or elute the sample. To obtain comparable HETP values among columns, the same conditions must be applied. Minimum theoretical plate values should be 1,000–3,000 plates/m for linear velocities of 50–600 cm/hr.

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 deionized water.
8. Equilibrate 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 (30–500 mM) may be used to elute bound protein from Nuvia™ IMAC Resin. As an alternative to imidazole elution, the pH can be lowered to within the range of 2.5–7.5. (**Note:** below pH 4, metal ions will be stripped off the medium.) 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 30–500 mM imidazole

Purification Protocol for a Packed Column

1. Equilibrate the column with at least 5 CV of binding buffer.
2. Apply the sample onto the column using the desired flow rate.
3. Wash the resin with at least 5 CV of wash buffer to remove unbound samples until the absorbance at 280 nm is at or near baseline.
4. Collect fractions 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 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 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 20 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. This may reduce the overall amount of target protein bound. However it will also increase 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 nonspecifically 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.

Regenerating the Medium

Regenerate metal-charged Nuvia IMAC Resins 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. Ensure that residual EDTA is completely removed from the column by washing it with 3–5 CV of binding buffer followed by 3–5 CV of distilled water. 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.

- 1 N NaOH up to 3 hr (removes precipitated, hydrophobic, and lipoproteins)
  - Exposure time is usually 1–3 hr
  - Rinse with 10 CV of distilled water

Alternatively, other cleaning-in-place solutions can be used.

- 2 M NaCl (removes ionic contaminants)
  - 10–15 min exposure time
  - Rinse with 10 CV of distilled water
- 70% ethanol or 30% isopropyl alcohol (removes precipitated, hydrophobic, and lipoproteins)
  - 15–20 min exposure time
  - Alternatively, use 0–30% gradient isopropyl alcohol over 5 CV, followed by 2 CV of 30% isopropyl alcohol
  - Rinse with 10 CV of distilled water

Remove cleaning solution(s) from column by rinsing with 10 CV of binding buffer (for example, 50 mM sodium phosphate, 300 mM NaCl, pH 8).

- Column cleaning can be monitored by UV signal. Ensure the eluate is at pH ~8 and the UV signal has returned to baseline

Sanitization

The column may be sanitized with 1 N NaOH.

Rinse solution from column with 3–5 CV of distilled water. Re-equilibrate the column with 3–5 CV of binding buffer.

Storage

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

- 2% benzyl alcohol
- 20% ethanol

Uncharged resin can be stored with 0.1 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
Insufficient amount of homogenization buffer	Dilute sample by adding more homogenization buffer
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
Clogs can also result from protein precipitation.	Consider the use of additives to improve protein stability and solubility
No protein is eluted	
Expression of target protein in extract is very low and is not found in the eluate	Check expression level of protein by estimating the amount in the extract, flowthrough, eluted fraction, and pellet upon centrifugation. Use western blotting (with anti-6x histidine antibodies and/or target protein–specific antibodies) ELISA, or enzyme activity determination
	Apply larger sample volume
	Minimize contact with hydrophobic surfaces (such as polystyrene tubes). Proteins at low concentration may bind to the surface of the tube
Target protein is found in inclusion bodies or possible insufficient lysis	Increase intensity/duration of disruption and homogenization
	If protein is insoluble, use 6 M guanidine HCl or 8 M urea to lyse denatured proteins
Target protein is found 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. A decrease in pH may result during the homogenization step or during growth of the culture medium. 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 has 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

Possible Cause	Solution
Protein precipitates during purification	
Temperature is too low	Perform the purification at room temperature
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 found 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 application
Target protein was detected in the flowthrough	Capillary sample loop is too small
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
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
Potential degradation of fusion protein by proteases	Include protease inhibitors in lysis buffer to reduce partial degradation. Consider performing purification at 4°C
Contaminants exhibit similar affinity to target protein	Add additional chromatography steps; that is, ion exchange, hydrophobic interaction, or size exclusion

Section 12
Ordering Information

Catalog #	Description*
Bottles	
7800800	**Nuvia IMAC Resin**, 25 ml bottle, Ni-charged
7800801	**Nuvia IMAC Resin**, 100 ml bottle, Ni-charged
7800802	**Nuvia IMAC Resin**, 500 ml bottle, Ni-charged
12003233	**Nuvia IMAC Resin**, 5 L, Ni-charged
12002782	**Nuvia IMAC Resin**, 10 L, Ni-charged
12004040	**Nuvia IMAC Resin**, 5 L
12004039	**Nuvia IMAC Resin**, 10 L
Prepackaged Formats	
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

Section 13
References

Bio-Rad (2016). Optimized resin for high productivity in downstream purification processes. Product information sheet. Bio-Rad Bulletin 6859.

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

Web site *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800*
Austria *43 1 877 89 01 177* ***Belgium*** *32 (0)3 710 53 00* ***Brazil*** *55 11 3065 7550*
Canada *1 905 364 3435* ***China*** *86 21 6169 8500*
Czech Republic *420 241 430 532* ***Denmark*** *45 44 52 10 00*
Finland *358 09 804 22 00* ***France*** *33 01 47 95 69 65* ***Germany*** *49 89 31 884 0*
Hong Kong *852 2789 3300* ***Hungary*** *36 1 459 6100* ***India*** *91 124 4029300*
Israel *972 03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *81 3 6361 7000*
Korea *82 2 3473 4460* ***Mexico*** *52 555 488 7670*
The Netherlands *31 (0)318 540 666* ***New Zealand*** *64 9 415 2280*
Norway *47 23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700*
Russia *7 495 721 14 04* ***Singapore*** *65 6415 3188*
South Africa *27 (0) 861 246 723* ***Spain*** *34 91 590 5200*
Sweden *46 08 555 12700* ***Switzerland*** *41 026 674 55 05*
Taiwan *886 2 2578 7189* ***Thailand*** *66 2 651 8311*
United Arab Emirates *971 4 8187300* ***United Kingdom*** *44 020 8328 2000*

D116698 Ver B US/EG *16-1212 0417 Sig 1216*