EconoFit Nuvia IMAC Uncharged Columns, 1 ml

Instruction Manual

Catalog number

12009289

Please read the instructions in this manual prior to using EconoFit Nuvia IMAC Uncharged Columns. If you have any questions or require any further assistance, please contact your Bio-Rad Laboratories representative.

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Section 1
Introduction

EconoFit Nuvia IMAC Uncharged Columns are convenient, disposable, prepacked low-pressure chromatography columns. EconoFit Columns offer both increased run-to-run uniformity and high purity of protein through the column design and novel resin technology. Compatible with aqueous buffers most commonly used for protein purification, EconoFit Columns offer improved performance for your protein separation needs.

Immobilized metal affinity chromatography (IMAC) is an excellent chromatography technique for purification of histidine-tagged proteins. The principle of IMAC is based on the affinity histidine has for metal ions. Side chains on the nitrilotriacetic acid (NTA) functional ligand selectively bind recombinant histidine-tagged proteins when the resin is charged with Ni^{2+} or other metals. The advantage of this technique is that proteins can often be purified close to homogeneity in a single step.

Nuvia IMAC Resin, a unique affinity support, is based on Bio-Rad's innovative UNOsphere Beads, which use proprietary polymerization and derivatization technologies.* UNOsphere technology enables the polymeric high-capacity IMAC resin to exhibit excellent flow properties without compromising protein binding, recovery, or purity.

Structural characteristics such as the polymeric nature, optimized ligand density, and open-pore structure of Nuvia IMAC Beads result in superb mechanical strength with high stringency, low nonspecific effects, and the ability to provide separations at fast flow rates. These unique features of the UNOsphere base matrix lend a number of performance benefits to the Nuvia IMAC Resin.

Nuvia IMAC is also stable across a wide pH range (2–14) and is compatible with most reagents commonly used in protein purifications, such as denaturants, detergents, and reducing agents. It is amenable to separations under native or denaturing conditions using liquid chromatography instrumentation, gravity flow columns, or sample-preparation spin columns.

Note: UNOsphere Media, from which Nuvia IMAC is derived, was designed to achieve the highest productivity (grams of drug or target per operational hour per liter of support) possible. UNOsphere Media may be run at the highest rates and loading capacities and will stay within the pressure limits of the column and chromatography system.

Under optimized conditions, the binding capacity for 6x histidine-tagged proteins is >40 mg/ml resin. The product is a 50% (v/v) slurry of resin, which is suspended in a 20% ethanol solution. Nuvia IMAC Resin is amenable to process- and laboratory-scale use and is available precharged with Ni^{2+} in bottles as well as prepacked into columns. Table 2 lists key characteristics of the resin, while Table 3 lists a variety of compounds compatible with Nuvia IMAC support.

Chemical Interactions

Nuvia IMAC Resin is composed of NTA groups coupled to a UNOsphere base matrix via a proprietary polymerization derivatization technology. It is well-suited to recombinant histidine-tagged purifications

* U.S. patent 6,423,666.

and results in high binding capacity and specificity for the target molecule. Although the most commonly used metal ion for histidine-tagged purifications is Ni^{2+}, other metals may be used to increase efficacy of purification. Choosing another type of immobilized metal ion can change the selectivity of an IMAC resin.

Section 2
Product Information

EconoFit Columns are disposable, easy-to-use, prepacked chromatographic columns supplied ready for use in convenient 1 and 5 ml sizes. They are quickly connected to liquid chromatography systems using 10-32 fittings. Columns are available for a variety of chromatographic techniques, including desalting (size exclusion [SEC]), ion exchange (IEX), affinity (AC), mixed-mode, and hydrophobic interaction chromatography (HIC). See Table 1 for specifications. Refer to **bio-rad.com/ResinsandColumns** for a complete listing of items in the EconoFit Column product line.

Table 1. EconoFit Nuvia IMAC Uncharged Column specifications.

Property	Description
Size	1 ml bed volume
Dimensions	25 mm length x 7 mm inner diameter
Recommended flow rate	1–2 ml/min (240–480 cm/hr)
Maximum flow rate	6 ml/min (1,440 cm/hr)
Fittings	10-32 (1/16"), female inlet and male outlet
Column material	Polypropylene
Frit material	High-density polyethylene
Shipping solution	20% ethanol
Storage conditions	20% ethanol
Autoclavability	Not autoclavable

Nuvia IMAC Uncharged Resin is also available in bottles. Refer to Ordering Information in section 11 of this manual. See Table 2 for specifications. Go to **bio-rad.com/ResinsandColumns** for more information.

Table 2. Nuvia IMAC Uncharged Resin specifications.

Property	Description
Functional ligand	Nitrilotriacetic acid
Base bead	UNOsphere
Particle size	38–53 µm
Mean particle size	50 µm
Metal ion capacity	≥18 µmol Cu^{2+}/ml
Dynamic binding capacity*	≥40 mg/ml
Recommended flow rate	480 cm/hr
Maximum operating pressure	45 psi
Chemical compatibility	See Table 3
Storage temperature	4°C to ambient temperature
Storage conditions	20% ethanol
Shelf life in 20% ethanol	>3 years at ambient temperature
Operational temperature	4–40°C
Autoclavability	Not autoclavable

* 10% determination of 1 mg/ml histidine-tagged pure protein (40 kD). **Note:** Dynamic binding capacity will vary from protein to protein.

Nuvia IMAC Columns are compatible with the aqueous buffers most commonly used with IMAC purification techniques (see Table 3).

Table 3. Chemical compatibilities for Nuvia IMAC Uncharged Columns.*

Reagent	Stability
Chelating Agent	
EDTA	1 mM
Sulfhydryl Reagents	
β-mercaptoethanol	20 mM
DTT	10 mM
TCEP	20 mM
Detergents	
Nonionic	2% (Triton X-100, Tween 20)
Zwitterionic	1% (CHAPS, CHAPSO)
Denaturing Agents	
Guanidine HCl	6 M
Urea	8 M
Other Additives	
NaCl	2 M (include at least 300 mM NaCl in buffers)
$MgCl_2$	100 mM (use HEPES to prevent precipitation)
$CaCl_2$	5 mM (use HEPES to prevent precipitation)
Glycerol	20% (backpressure may increase significantly, slower flow rates may be required)
Ethanol	20%

* Nuvia IMAC Resin binding capacities are unaffected up to the concentrations given when employing typical reagents used for histidine-tagged protein purification.

Section 3
Immobilizing Metal Ions

Efficacy of protein binding by IMAC depends on two factors: the number of available histidine, cysteine, and tryptophan residues on a protein's surface and the number of coordination sites on the immobilized ion that are not occupied by the chelating ligand and thus available to bind amino acid residues. Nuvia IMAC Resin uses a quadridentate ligand (NTA), which leaves two of the six coordination sites on the nickel ion accessible to the protein of interest.

Although the most commonly used ion is Ni^{2+}, protein selectivity may be increased through the choice of metal ion used, understanding of the structure of the metal-chelate complex and its interaction with the protein, knowledge of the protein's expression level, and the ligand density of the IMAC adsorbent. While high ligand density usually means higher binding capacity, it can also translate into lower target protein selectivity. Nuvia IMAC Resin, based on the polymeric UNOsphere technology, has been specifically formulated with an optimal number of chelating ligands on the resin's surface and pores to deliver both good capacity and excellent protein purity.

1. Equilibrate the column with 5 column volumes (CV) of 50 mM sodium acetate, 0.3 M NaCl, pH 4.0.
2. After slurry packing is complete, the column is ready for the removal or addition of metal ions.
3. If necessary, strip any metal ions by washing with 10 CV of 50 mM sodium phosphate, 0.3 M NaCl, and 0.05–0.5 M EDTA, pH 7.5.
4. Make a 0.1–0.3 M solution of the metal ion of choice. For best results, the pH of the solution should be <7 (neutral to weakly acidic).
5. Apply 3–5 CV of the metal ion solution.
6. Wash with 5 CV of 50 mM sodium acetate, 0.3 M NaCl, pH 4.0. Remove excess ions by washing.
7. Wash with 10 CV of deionized water.
8. Equilibrate with at least 5 CV of starting buffer; for example, 50 mM sodium phosphate, 0.3 M NaCl, pH 7–8. The column is now ready for separation.

Section 4
Buffers and Methods

IMAC methods can be run using either native or denaturing purification protocols. Under native conditions, proteins are purified using buffers that help retain the natural folded structure of the target protein. Under denaturing conditions, strong chaotropic agents (typically 6–8 M urea or 6 M guanidine) are added to the buffers, allowing target proteins to be purified in their unfolded states. The recommended buffer compositions and formulations are provided in Table 4.

Table 4. Suggested buffer composition.

Step	Buffer*			
	NaCl, mM	Na Phosphate, mM	Imidazole, mM	Urea, M
Native lysis/wash buffer 1	300	50	5	NA
Native wash buffer 2	300	50	25	NA
Native elution buffer	300	50	500	NA
Denaturing lysis/wash buffer 1	300	50	5	6
Denaturing wash buffer 2	300	50	25	6
Denaturing elution buffer	300	50	500	6

* For all buffer formulations, adjust pH to 8.0 with KOH or H_3PO_4 and filter through a 0.2 µm filter. Native buffers can be stored up to 1 year at 4–22°C. Denaturing buffers must be made fresh and used within 7 days or frozen in aliquots at –20°C for later use.

Section 5
Quick Solubility Screening Protocols

Before choosing a native or denaturing purification protocol, it is useful to determine both the approximate expression level of a protein and whether the overexpressed target protein partitions into the soluble or insoluble fraction. Soluble proteins are typically purified with the native purification procedure while insoluble proteins must be solubilized in stringent denaturants (urea or guanidine) and are purified with the denaturing procedure.

The following procedure provides a quick screen for solubility and expression level:

1. Pellet ~2 ml of *Escherichia coli* culture by centrifugation at 4,000 x g for 10 min at 4°C.
2. Resuspend the pellet in 500 µl of phosphate buffered saline (PBS) and sonicate on ice for 60 sec, in 10 sec pulses. Remove 50 µl of sonicate and label the sample Total. Centrifuge the lysate at 12,000 x g for 10 min at 4°C. Transfer the supernatant to a clean tube. Remove 50 µl of the supernatant and label the tube Soluble.
3. Resuspend the insoluble pellet in 500 µl of 6 M urea in 1x PBS and sonicate on ice for 60 sec, in 10 sec pulses. Centrifuge the lysate at 12,000 x g for 10 min at 4°C. Remove 50 µl of the supernatant and label the tube Insoluble.
4. To each of the 50 µl samples, add 150 µl of Laemmli buffer and boil for 5 min at 95°C.
5. Load 10 µl of each sample on an SDS-PAGE gel.
6. Examine the soluble and insoluble fractions for the target protein. Approximate the expression level and determine partitioning of the target protein.

Section 6
Preparation of *E. coli* Lysates

For *E. coli* cultures expressing medium to high levels of histidine-tagged proteins (≥10% of total protein), 200 ml of culture will yield sufficient material for a 1 ml column purification, and 1,000 ml of culture will yield sufficient material for a 5 ml column purification run. For cultures expressing protein at low levels (~10% of total protein), the culture volumes will need to be determined empirically for each protein.

Native Lysates

1. Harvest the cell pellet by centrifugation at 8,000 x g for 10 min at 4°C.
2. Determine the weight of the pellet and resuspend in 10 culture volumes of native lysis/wash buffer 1 (200 ml of culture typically yields 0.8 g of paste and results in 8 ml of lysate).
3. Sonicate the lysate on ice four times at 1 min intervals.
4. Centrifuge the lysate at 12,000 x g for 20 min at 4°C.
5. Remove the supernatant and filter it through a 0.2 µm filter immediately before applying to the column.

Denatured Lysates

1. Harvest the cell pellet by centrifugation at 8,000 x g for 10 min at 4°C.
2. Determine the weight of the pellet and resuspend in 10 culture volumes of denaturing lysis/wash buffer 1 (200 ml of culture typically yields 0.8 g of paste and results in 8 ml of lysate).
3. Sonicate the lysate four times at 1 min intervals.
4. Centrifuge the lysate at 12,000 x g for 20 min at 4°C.
5. Remove the supernatant and filter it through a 0.2 µm filter immediately before applying to the column.

Section 7
Preparing a Column and Subsequent Purification

Prepare buffer sets for either the native or denaturing purification protocols using a single buffer set throughout the procedure. To prepare the column for the procedure, remove the top closure and connect the column to the chromatography system. Open the bottom closure and connect the column outlet to the system. Flush the packing solution (20% ethanol) from the column by running 2 CV of water at a flow rate of 2 ml/min. The column is now ready for the purification steps (see Table 5).

Table 5. Purification method suggestions.

Step	Column Volumes, CV	Flow Rate, ml/min
Equilibrate	5	2
Lysate load	Varies based on sample volume	1*
Wash 1	6	1
Wash 2	6	2
Elute	5	2

* Depending on sample viscosity.

Standard methods that are compatible with any type of chromatography system are listed in the following steps. To maximize binding capacity with large proteins (>100 kD), for purification at 4°C, or for purifications under denaturing conditions, the lysate load flow rate can be decreased to 0.5 ml/min. Whether this decrease maximizes flow rate will have to be determined empirically for individual proteins.

1. Equilibrate the column with 5 CV of equilibration/wash buffer 1 at 2 ml/min.
2. Load the sample lysate at 1 ml/min.
3. Wash the column with 6 CV of wash buffer 1 at 1 ml/min.
4. Wash the column with 6 CV of wash buffer 2 at 2 ml/min.

   Note: Equivalent to 5% buffer B/elution buffer wash.
5. Elute the purified protein with 10 CV of elution buffer at 2 ml/min.
6. Prior to quantitation of the protein concentration, the purified protein should be exchanged into a nonimidazole buffer (imidazole can absorb at 280 nm). Purified protein from denaturing purifications should be exchanged into another buffer through dialysis.

The chromatogram in Figure 1 illustrates a representative purification of a high-expressing soluble protein purified using the native buffer set and method described in Table 5.

Note: IMAC buffers made with potassium salts are more stable than sodium salt–based buffers. However, potassium will complex with sodium dodecyl sulfate (SDS) in Laemmli buffer and precipitate out of solution. Prior to analyzing IMAC samples on gels, the samples must be diluted at least 1:7 with Laemmli buffer to prevent precipitation.

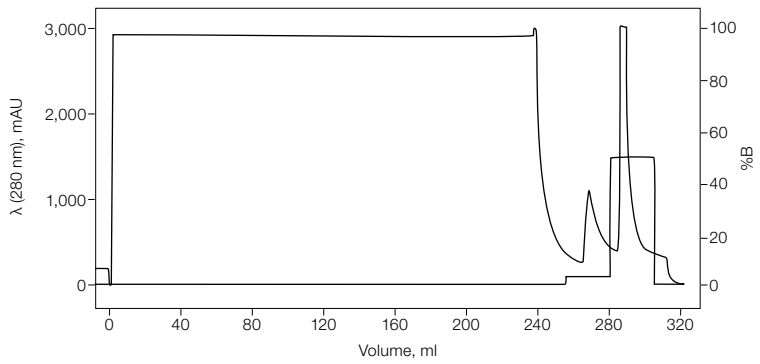

Fig. 1. Typical IMAC purification. A histidine-tagged protein was purified from the soluble fraction using the standard Nuvia IMAC native purification protocol. Clarified *E. coli* lysate was loaded onto a 5 ml Nuvia IMAC Ni-Charged Column. The column was washed with 6 CV of wash buffer 1 followed by 6 CV of wash buffer 2. Purified protein was eluted with 5 CV of elution buffer.

Section 8
Scaling Up

EconoFit Columns are available in 1 and 5 ml formats. The Nuvia IMAC Resin is also available in various amounts, from 25 ml bottles to larger bulk quantities, for scaling up methods developed using the columns. For quick scale-up, two or three columns of the same type can be connected in series, so take care to maintain an overall system pressure ≤45 psi.

In addition, Bio-Rad carries an extensive line of empty chromatography columns from laboratory to process scale. Ask your local Bio-Rad representative or go to **bio-rad.com/ResinsandColumns** for more information.

Section 9
Regenerating, Cleaning, Sanitizing, and Storing Columns

Protein cross-contamination, frit clogging, and increased backpressure can result from running a column beyond the recommended number of uses. After repeated use, a column may run slower or produce high backpressure, a result that should be expected due to the nature of the sample mixture. The following cleaning and regeneration procedures may be used. However, we recommend that you dispose of a column after several uses. To avoid cross-contamination, we recommend that each column be designated for a single protein. To maintain good flow properties, we recommend that the columns be cleaned between uses.

Run the cleaning protocol at 2 ml/min for 1 ml columns.

High Salt/Acid Cleaning

1. Rinse the column with 2 CV of water at 2 ml/min.
2. Wash the column with 5 CV of 500 mM NaCl, 50 mM Tris, pH 8.0 at 2 ml/min.
3. Wash the column with 5 CV of 500 mM NaCl, 100 mM NaOAc, pH 4.5 at 2 ml/min.
4. Rinse the column with 2 CV of water at 2 ml/min.
5. Store the column in 20% ethanol at 4°C.

Chaotropic Cleaning

1. Rinse the column with 2 CV of water at 2 ml/min.
2. Wash the column with 5 CV of 6 M guanidine HCl at 2 ml/min.
3. Wash the column with 2 CV of water at 2 ml/min.
4. Rinse the column with 2 CV of water at 2 ml/min.
5. Store the column in 20% ethanol at 4°C.

Other Cleaning Guidelines

In situations where it is desirable to run different proteins over the same column, completely sanitize, strip, and recharge the column between sample runs. Take care when handling and disposing of metal-containing solutions.

1. Clean the column with 10 CV of 1 M NaOH.
2. Rinse the column with 10 CV of water.
3. Strip metal ions with 5 CV of 0.1 M EDTA.
4. Rinse the column with 10 CV of water.
5. Recharge the column with 5 CV of 0.1 M nickel sulfate, pH 4.5.
6. Rinse the column with 10 CV of water.
7. Store the column in 20% ethanol.

Section 10
Troubleshooting Guide

Possible Causes	Possible Solutions
Column Clogging or Slow Flow Rate	
Particulates in sample	Filter all samples and buffers through 0.2 µm filter prior to application
Sample too viscous	Add nuclease to lysate to degrade DNA. Centrifuge and filter lysate again
No Target Protein in Eluate	
Low level of target	Check expression level of protein in starting SDS-PAGE material
Target protein not binding	▪ Check levels of target protein in lysate, flowthrough, wash fractions, and eluted fractions ▪ Check for presence of histidine tag with antihistidine antibody
Target Protein in Flowthrough	
Histidine tag not accessible	▪ Purify protein under denaturing conditions to expose histidine tag ▪ Reclone histidine tag onto opposite terminus (N- or C- terminus)
Proteolysis and removal	Include protease inhibitors in histidine-tagged lysis buffer or purify in the cold
Precipitation during Purification	
Binding capacity of column exceeded	Load less sample
Protein aggregating	▪ Include low amount of detergent (0.1% Triton X-100, Tween 20) ▪ Include glycerol up to 10%
Protein too concentrated in step elution	Elute with imidazole gradient
Eluted Protein Is Impure	
Contaminants coeluting	▪ Elute with imidazole gradient (10–500 mM) rather than step elution ▪ Increase imidazole in the wash to increase wash stringency, but keep below 40 mM
Target Protein Is Degraded	
Proteolysis of target	▪ Add protease inhibitors to protein lysate ▪ Purify at 4°C or under denaturing conditions

Section 11
Ordering Information

Catalog #	Description
12009289	**EconoFit Nuvia IMAC Uncharged Column**, 1 x 1 ml column
12004040	**Nuvia IMAC Uncharged Resin**, 5 L bottle
12004039	**Nuvia IMAC Uncharged Resin**, 10 L bottle

Section 12
Bibliography

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